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(54) Title: SYNTHETIC PEPTIDE CONSTRUCTS FOR THE DIAGNOSIS AND TREATMENT OF PERIODONTITIS ASSOCIATED WITH *PORPHYROMONAS GINGIVALIS*

(57) Abstract

The present invention relates to an oral composition and an immunogenic composition for the suppression of the pathogenic effects of the intra-oral bacterium *Porphyromonas gingivalis* associated with periodontal disease. The invention also relates to methods of treating or reducing the prospect of *P. gingivalis* infection.

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Synthetic Peptide Constructs for the Diagnosis and Treatment of Periodontitis associated with *Porphyromonas gingivalis*

FIELD OF THE INVENTION

This invention relates to an oral composition and an immunogenic composition for the suppression of the pathogenic effects of the intra-oral bacterium *Porphyromonas gingivalis* associated with periodontal disease. It also relates to diagnostic tests for the presence of *Porphyromonas gingivalis* in subgingival plaque samples and specific antibodies against *P. gingivalis* antigens in sera. The compositions comprise synthetic peptide constructs corresponding to structurally and functionally significant areas of the PrtR-PrtK proteinase-adhesin complex of *Porphyromonas gingivalis*. Also disclosed are methods for preparing the synthetic peptide constructs. The synthetic peptide constructs are useful as immunogens in raising an immune response against *P. gingivalis* and can be used to generate protein-specific and peptide-specific antisera useful for passive immunization and as reagents for diagnostic assays.

BACKGROUND OF THE INVENTION

Periodontal diseases are bacterial-associated inflammatory diseases of the supporting tissues of the teeth and range from the relatively mild form of gingivitis, the non-specific, reversible inflammation of gingival tissue to the more aggressive forms of periodontitis which are characterised by the destruction of the tooth's supporting structures. Periodontitis is associated with a subgingival infection of a consortium of specific Gram-negative bacteria that leads to the destruction of the periodontium and is a major public health problem. One bacterium that has attracted considerable interest is *Porphyromonas gingivalis* as the recovery of this microorganism from adult periodontitis lesions can be up to 50% of the subgingival anaerobically cultivable flora, whereas *P. gingivalis* is rarely recovered, and then in low numbers, from healthy sites. A proportional increase in the level of *P. gingivalis* in subgingival plaque has been associated with an increased severity of periodontitis and eradication of the microorganism from the cultivable subgingival microbial population is accompanied by resolution of the disease. The progression of periodontitis lesions in non-human primates has been demonstrated with the subgingival implantation of *P. gingivalis*.

These findings in both animals and humans suggest a major role for *P. gingivalis* in the development of adult periodontitis.

P. gingivalis is a black-pigmented, anaerobic, proteolytic Gram-negative rod that obtains energy from the metabolism of specific amino acids. The microorganism has an absolute growth requirement for iron, preferentially in the form of heme or its Fe(III) oxidation product hemin and when grown under conditions of excess hemin is highly virulent in experimental animals. A number of virulence factors have been implicated in the pathogenicity of *P. gingivalis* including the capsule, adhesins, cytotoxins and extracellular hydrolytic enzymes. In order to develop an efficacious and safe vaccine to prevent *P. gingivalis* colonisation it is necessary to identify effective antigens that are involoved in virulence that have utility as immunogens to generate neutralising antibodies.

We have purified and characterised a 300 kDa multiprotein complex of cysteine proteinases and adhesins which is a major virulence factor for *P. gingivalis*. This complex was biochemically characterised and disclosed in International Patent Application No. PCT/AU96/00673, the disclosure of which is incorporated herein by reference. The complex consists of a 160 kDa Arg-specific proteinase with C-terminal adhesin domains (designated PrtR) associated with a 163 kDa Lys-specific proteinase also with C-terminal adhesin domains (designated PrtK). The C-terminal adhesin domains of the PrtR and PrtK have homology with an haemagglutinin from *P. gingivalis* designated HagA. The gene encoding HagA has been disclosed in the international patent WO96/17936, the disclosure of which is incorporated herein by reference.

SUMMARY OF THE INVENTION

The present inventors have identified a number of structurally and functionally significant sequences from the 300 kDa multiprotein complex of cysteine proteinases and adhesins which is a major virulence factor for *P. gingivalis*. These sequences are set out in Table 1.

Table 1. Amino acid sequences of the PrtR-PrtK proteinase-adhesin complex of functional significance.

Proteinase Active Site	Sequence (single letter code)	Designation
PrtR45 (426-446)	FNGGISLANYTGHGSETAWGT	PAS1(R45)
PrtK48 (432-453)	LNTGVSFANYTAHGSETAWADP	PAS1(K48)
PrtR45 (467-490)	FDVACVNGDFLFSMPCFAEALMRA	PAS2(R45)
PrtK48 (473-496)	IGNCCITAQFDYVQPCFGEVITRV	PAS2(K48)
Adhesin Binding Motif	**Sequence (single letter code)**	**Designation**
PrtR45 (660-689)	GEPNPYQPVSNLTATTQGQKVTLKWDAPSTK	ABM1(R45)
PrtR44 (919-949)	EGSNEFAPVQNLTGSAVGQKVTLKWDAPNGT	ABM1(R44)
PrtR17 (1375-1405)	VNSTQFNPVKNLKAQPDGGDVVLKWEAPSAK	ABM1(R17)
PrtK48 (681-711)	GEPSPYQPVSNLTATTQGQKVTLKWEAPSAK	ABM1(K48)
PrtK39 (940-970)	EGSNEFAPVQNLTGSSVGQKVTLKWDAPNGT	ABM1(K39)
PrtK44 (1393-1425)	VNSTQFNPVQNLTAEQAPNSMDAILKWNAPASK	ABM1(K44)
HagA (1837-1863)	QFNPVQNLTGSAVGQKVTLKWDAPNGT	ABM1(HagA1)
HagA (1381-1407)	QFNPVQNLTGSAVGQKVTLKWDAPNGT	ABM1(HagA2)
HagA (925-951)	QFNPVQNLTGSAVGQKVTLKWDAPNGT	ABM1(HagA3)
HagA (474-499)	FAHVQNLTGSAVGQKVTLKWDAPNGT	ABM1(HagA4)
HagA (202-227)	FAPVQNLQWSVSGQTVTLTWQAPASD	ABM1(HagA5)
HagA (2293-2321)	QFNPVQNLTAEQAPNSMDAILKWNAPASK	ABM1(HagA6)
PrtR44 (865-893)	DYTYTVYRDGTKIKEGLTATTFEEDGVAT	ABM2(R44)
PrtR17 (1322-1350)	DYTYTVYRDGTKIKEGLTETTFEEDGVAT	ABM2(R17)
PrtR27 (1580-1608)	SYTYTVYRDGTKIKEGLTETTYRDAGMSA	ABM2(R27)
PrtK39 (886-914)	SYTYTVYRDGTKIKEGLTATTFEEDGVAA	ABM2(K39)
PrtK44 (1340-1368)	DYTYTVYRDGTKIKEGLTETTFEEDGVAT	ABM2(K44A)
PrtK44 (1606-1634)	SYTYTIYRNNTQIASGVTETTYRDPDLAT	ABM2(K44B)
HagA (2236-2264)	DYTYTVYRDGTKIKEGLTETTFEEDGVAT	ABM2(HagA1)
HagA (1780-1808)	DYTYTVYRDGTKIKEGLTETTFEEDGVAT	ABM2(HagA2)
HagA (1324-1352)	DYTYTVYRDGTKIKEGLTETTFEEDGVAT	ABM2(HagA3)
HagA (868-896)	DYTYTVYRDGTKIKEGLTETTFEEDGVAT	ABM2(HagA4)
HagA (415-443)	DYTYTVYRDNVVIAQNLAATTFNQENVAP	ABM2(HagA5)
HagA (2502-2530)	SYTYTIYRNNTQIASGVTETTYRDPDLAT	ABM2(HagA6)
PrtR44 (946-971)	PNGTPNPNPNPNPNPNPGTTTLSESF	ABM3(R44)
PrtK39 (967-989)	PNGTPNPNPNPNPNPGTTLSESF	ABM3(K39)
HagA (1860-1881)	PNGTPNPNPNPNPGTTTLSESF	ABM3(HagA1)
HagA (1404-1425)	PNGTPNPNPNPNPGTTTLSESF	ABM3(HagA2)
HagA (948-969)	PNGTPNPNPNPNPGTTTLSESF	ABM3(HagA3)
HagA (496-513)	PNGTPNPNPGTTTLSESF	ABM3(HagA4)
PrtR17 (1278-1297)	WIERTVDLPAGTKYVAFRHY	ABM4(R17)
PrtR44 (1028-1043)	WRQKTVDLPAGTKYVAFRHF	ABM4(R44)
PrtK44 (1296-1315)	WIERTVDLPAGTKYVAFRHY	ABM4(K44A)
PrtK44 (1565-1584)	WRQKTVDLPAGTKYVAFRHF	ABM4(K44B)
PrtK39 (1116-1135)	WYQKTVQLPAGTKYVAFRHF	ABM4(K39)
HagA (2191-2211)	WIERTVDLPAGTKYVAFRHY	ABM4(HagA1)
HagA (1736-1755)	WIERTVDLPAGTKYVAFRHY	ABM4(HagA2)
HagA (1280-1299)	WIERTVDLPAGTKYVAFRHY	ABM4(HagA3)
HagA (824-843)	WIERTVDLPAGTKYVAFRHY	ABM4(HagA4)
HagA (2012-2031)	WYQKTVQLPAGTKYVAFRHF	ABM4(HagA5)
HagA (1556-1575)	WYQKTVQLPAGTKYVAFRHF	ABM4(HagA6)

Table 1. Continued,

Adhesin Binding Motif	Sequence (single letter code)	Designation
HagA (2461-2480)	WYQKTVQLPAGTKYVAFRHF	ABM4 (HagA7)
HagA (1100-1119)	WYQKTVQLPAGTKYVAFRHF	ABM4 (HagA8)
HagA (644-663)	WYQKTVQLPAGTKYVAFRHF	ABM4 (HagA9)
HagA (372-392)	ERTIDLSAYAGQQVYLAFRHF	ABM4 (HagA10)
PrtR15 (1154-1169)	PAEWTTIDADGDGQGW	ABM5 (R15)
PrtR44 (976-991)	PASWKTIDADGDGHGW	ABM5 (R44)
PrtK15 (1172-1187)	PAEWTTIDADGDGQGW	ABM5 (K15)
PrtK39 (994-1009)	PASWKTIDADGDGHGW	ABM5 (K39)
PrtK44 (1439-1454)	PASWKTIDADGDGNNW	ABM5 (K44)
HagA (2068-2083)	PAEWTTIDADGDGQGW	ABM5 (HagA1)
HagA (1612-1627)	PAEWTTIDADGDGQGW	ABM5 (HagA2)
HagA (1156-1171)	PAEWTTIDADGDGQGW	ABM5 (HagA3)
HagA (700-715)	PAEWTTIDADGDGQGW	ABM5 (HagA4)
HagA (1430-1445)	PASWKTIDADGDGNNW	ABM5 (HagA5)
HagA (974-989)	PASWKTIDADGDGNNW	ABM5 (HagA6)
HagA (1886-1901)	PASWKTIDADGDGNNW	ABM5 (HagA7)
HagA (518-533)	PASWKTIDADGDGNNW	ABM5 (HagA8)
HagA (2335-2350)	PSSWKTIDADGDGNNW	ABM5 (HagA9)
HagA (243-258)	PNGWTMIDADGDGHNW	ABM5 (HagA10)
PrtR44 (919-938)	EGSNEFAPVQNLTGSAVGQK	ABM6 (R44)
PrtR45 (659-678)	GEPNPYQPVSNLTATTQGQK	ABM6 (R45)
PrtK39 (940-959)	EGSNEFAPVQNLTGSSVGQK	ABM6 (K39)
PrtK48 (681-700)	GEPSPYQPVSNLTATTQGQK	ABM6 (K48)
PrtK44 (1394-1412)	NSTQFNPVQNLTAEQAPNS	ABM6 (K44)
HagA (469-488)	EGSNEFAHVQNLTGSAVGQK	ABM6 (HagA1)
HagA (1834-1852)	DPVQFNPVQNLTGSAVGQK	ABM6 (HagA2)
HagA (1378-1396)	DPVQFNPVQNLTGSAVGQK	ABM6 (HagA3)
HagA (922-940)	DPVQFNPVQNLTGSAVGQK	ABM6 (HagA4)
HagA (197-216)	EGGNEFAPVQNLQWSVSGQT	ABM6 (HagA5)
HagA (2290-2308)	NPTQFNPVQNLTAEQAPNS	ABM6 (HagA6)
PrtR44 (894-918)	GNHEYCVEVKYTAGVSPKVCKDVTV	ABM7 (R44)
PrtR17 (1351-1375)	GNHEYCVEVKYTAGVSPKKCVNVTV	ABM7 (R17)
PrtR27 (1610-1630)	SHEYCVEVKYTAGVSPKVCVD	ABM7 (R27)
PrtK39 (915-939)	GNHEYCVEVKYTAGVSPKVCKDVTV	ABM7 (K39)
PrtK44 (1369-1393)	GNHEYCVEVKYTAGVSPKKCVNVTV	ABM7 (K44)
HagA (2265-2289)	GNHEYCVEVKYTAGVSPKVCVNVTI	ABM7 (Hag1)
HagA (444-468)	GQYNYCVEVKYTAGVSPKVCKDVTV	ABM7 (Hag2)
HagA (1809-1833)	GNHEYCVEVKYTAGVSPEVCVNVTV	ABM7 (Hag3)
HagA (1353-1377)	GNHEYCVEVKYTAGVSPEVCVNVTV	ABM7 (Hag4)
HagA (897-921)	GNHEYCVEVKYTAGVSPEVCVNVTV	ABM7 (Hag5)

Accordingly in a first aspect the present invention consists in a composition for use in raising an immune response against *Porphyromonas gingivalis*, the composition including a suitable adjuvant and/or acceptable carrier or excipient and at least one peptide selected from the group consisting of:-

FNGGISLANYTGHGSETAWGT;
LNTGVSFANYTAHGSETAWADP;
FDVACVNGDFLFSMPCFAEALMRA;
IGNCCITAQFDYVQPCFGEVITRV;
GEPNPYQPVSNLTATTQGQKVTLKWDAPSTK;
EGSNEFAPVQNLTGSAVGQKVTLKWDAPNGT;
VNSTQFNPVKNLKAQPDGGDVVLKWEAPSAK;
GEPSPYQPVSNLTATTQGQKVTLKWEAPSAK;
EGSNEFAPVQNLTGSSVGQKVTLKWDAPNGT;
VNSTQFNPVQNLTAEQAPNSMDAILKWNAPASK;
QFNPVQNLTGSAVGQKVTLKWDAPNGT;
FAHVQNLTGSAVGQKVTLKWDAPNGT;
FAPVQNLQWSVSGQTVTLTWQAPASD;
QFNPVQNLTAEQAPNSMDAILKWNAPASK;
DYTYTVYRDGTKIKEGLTATTFEEDGVAT;
DYTYTVYRDGTKIKEGLTETTFEEDGVAT;
SYTYTVYRDGTKIKEGLTETTYRDAGMSA;
SYTYTVYRDGTKIKEGLTATTFEEDGVAA;
DYTYTVYRDGTKIKEGLTETTFEEDGVAT;
SYTYTIYRNNTQIASGVTETTYRDPDLAT;
DYTYTVYRDNVVIAQNLAATTFNQENVAP;
SYTYTIYRNNTQIASGVTETTYRDPDLAT;
PNGTPNPNPNPNPNPNPGTTTLSESF;
PNGTPNPNPNPNPNPGTTLSESF;
PNGTPNPNPNPNPGTTTLSESF;
PNGTPNPNPGTTTLSESF;
WIERTVDLPAGTKYVAFRHY;
WRQKTVDLPAGTKYVAFRHF;
WYQKTVQLPAGTKYVAFRHF;
ERTIDLSAYAGQQVYLAFRHF;
PAEWTTIDADGDGQGW;
PASWKTIDADGDGHGW;
PASWKTIDADGDGNNW;
PSSWKTIDADGDGNNW;
PNGWTMIDADGDGHNW;
EGSNEFAPVQNLTGSAVGQK;

GEPNPYQPVSNLTATTQGQK;
EGSNEFAPVQNLTGSSVGQK;
GEPSPYQPVSNLTATTQGQK;
NSTQFNPVQNLTAEQAPNS;
EGSNEFAHVQNLTGSAVGQK;
DPVQFNPVQNLTGSAVGQK;
EGGNEFAPVQNLQWSVSGQT;
NPTQFNPVQNLTAEQAPNS;
GNHEYCVEVKYTAGVSPKVCKDVTV;
GNHEYCVEVKYTAGVSPKKCVNVTV;
SHEYCVEVKYTAGVSPKVCVD;
GNHEYCVEVKYTAGVSPKKCVNVTV;
GNHEYCVEVKYTAGVSPKVCVNVTI;
GQYNYCVEVKYTAGVSPKVCKDVTV; and
GNHEYCVEVKYTAGVSPEVCVNVTV.

In a preferred embodiment of the first aspect of the present invention, the composition includes at least one peptide selected from the group consisting of:-

FNGGISLANYTGHGSETAWGT;
LNTGVSFANYTAHGSETAWADP;
PYQPVSNLTATTQGQKVTLKWDAPSTK;
SYTYTVYRDGTKIKEGLTATTFEEDGVAA;
VTLKWDAPNGTPNPNPNPNPNPNPGTTTLSESF;
WIERTVDLPAGTKYVAFRHY;
PAEWTTIDADGDGQGW; and
EGSNEFAPVQNLTGSAVGQK.

Where the composition includes more than one peptide the peptides may be present in the composition as individual peptides or in multimeric forms. Where multimeric forms are used the multimer may comprise multiple copies of the same peptide, however, it is preferred that the multimer includes differing peptides.

Peptides (PAS1 and PAS2) of Table 1 represent sequences of the Arg-specific (PrtR45) and Lys-specific (PrtK48) cysteine proteinases which form the active site containing the Cys-His catalytic dyad.

The remaining peptides (ABM peptides) represent adhesin binding motifs of the PrtR-PrtK protein-adhesin complex and HagA and together with the proteinase active site sequences, have proven to be effective as synthetic peptide vaccines.

In a second aspect the present invention consists in a peptide, the peptide being selected from the group consisting of:-

FNGGISLANYTGHGSETAWGT;
LNTGVSFANYTAHGSETAWADP;
FDVACVNGDFLFSMPCFAEALMRA;
IGNCCITAQFDYVQPCFGEVITRV;
GEPNPYQPVSNLTATTQGQKVTLKWDAPSTK;
EGSNEFAPVQNLTGSAVGQKVTLKWDAPNGT;
VNSTQFNPVKNLKAQPDGGDVVLKWEAPSAK;
GEPSPYQPVSNLTATTQGQKVTLKWEAPSAK;
EGSNEFAPVQNLTGSSVGQKVTLKWDAPNGT;
VNSTQFNPVQNLTAEQAPNSMDAILKWNAPASK;
QFNPVQNLTGSAVGQKVTLKWDAPNGT;
FAHVQNLTGSAVGQKVTLKWDAPNGT;
FAPVQNLQWSVSGQTVTLTWQAPASD;
QFNPVQNLTAEQAPNSMDAILKWNAPASK;
DYTYTVYRDGTKIKEGLTATTFEEDGVAT;
DYTYTVYRDGTKIKEGLTETTFEEDGVAT;
SYTYTVYRDGTKIKEGLTETTYRDAGMSA;
SYTYTVYRDGTKIKEGLTATTFEEDGVAA;
DYTYTVYRDGTKIKEGLTETTFEEDGVAT;
SYTYTIYRNNTQIASGVTETTYRDPDLAT;
DYTYTVYRDNVVIAQNLAATTFNQENVAP;
SYTYTIYRNNTQIASGVTETTYRDPDLAT;
PNGTPNPNPNPNPNPNPGTTTLSESF;
PNGTPNPNPNPNPNPGTTLSESF;
PNGTPNPNPNPNPGTTTLSESF;
PNGTPNPNPGTTTLSESF;
WIERTVDLPAGTKYVAFRHY;
WRQKTVDLPAGTKYVAFRHF;
WYQKTVQLPAGTKYVAFRHF;

ERTIDLSAYAGQQVYLAFRHF;
PAEWTTIDADGDGQGW;
PASWKTIDADGDGHGW;
PASWKTIDADGDGNNW;
PSSWKTIDADGDGNNW;
PNGWTMIDADGDGHNW;
EGSNEFAPVQNLTGSAVGQK;
GEPNPYQPVSNLTATTQGQK;
EGSNEFAPVQNLTGSSVGQK;
GEPSPYQPVSNLTATTQGQK;
NSTQFNPVQNLTAEQAPNS;
EGSNEFAHVQNLTGSAVGQK;
DPVQFNPVQNLTGSAVGQK;
EGGNEFAPVQNLQWSVSGQT;
NPTQFNPVQNLTAEQAPNS;
GNHEYCVEVKYTAGVSPKVCKDVTV;
GNHEYCVEVKYTAGVSPKKCVNVTV;
SHEYCVEVKYTAGVSPKVCVD;
GNHEYCVEVKYTAGVSPKKCVNVTV;
GNHEYCVEVKYTAGVSPKVCVNVTI;
GQYNYCVEVKYTAGVSPKVCKDVTV; and
GNHEYCVEVKYTAGVSPEVCVNVTV.

In a preferred embodiment of the second aspect of the present invention, the peptide is selected from the group consisting of:-

FNGGISLANYTGHGSETAWGT;
LNTGVSFANYTAHGSETAWADP;
PYQPVSNLTATTQGQKVTLKWDAPSTK;
SYTYTVYRDGTKIKEGLTATTFEEDGVAA;
VTLKWDAPNGTPNPNPNPNPNPNPGTTTLSESF;
WIERTVDLPAGTKYVAFRHY;
PAEWTTIDADGDGQGW; and
EGSNEFAPVQNLTGSAVGQK.

As will be readily apparent to persons skilled in this area these peptides may be used as antigens in diagnostic tests or as immunogens in formulations.

In a third aspect the present invention consists in an antibody preparation comprising antibodies specifically directed against the composition of the first aspect of the invention or the peptides of the second aspect of the invention. The antibodies may be either polyclonal or monoclonal antibodies.

In a fourth aspect the present invention consists in a method of treating a subject suffering from *Porphyromonas gingivalis* infection, the method comprising administering to the subject an effective amount of the antibody preparation of the third aspect.

In a preferred embodiment the antibody preparation is administered as a mouth wash or as a dentifrice.

In a fifth aspect the present invention consists in a method of reducing the prospect of *P. gingivalis* infection in an individual and/or severity of disease, the method comprising administering to the individual an amount of the composition of the first aspect effective to induce an immune response in the individual directed against *P. gingivalis*.

Peptides can be synthesized using one of the several methods of peptide synthesis known in the art including standard solid phase peptide synthesis using t-butyloxycarbonyl amino acids (Mitchell et al., 1978, J. Org. Chem. 43:2845-2852) using 9-fluorenylmethyloxycarbonyl (Fmoc) amino acids on a polyamide support (Druland et al., 1986, J. Chem. Soc.Perkin Trans. 1 125-137) by pepscan synthesis (Geysen et al., 1987, J. Immunol Methods 03:259; 1984, Proc. Natl. Acad. Sci. USA, 81:3998) or by standard liquid phase synthesis.

A variety of methods for the synthesis of multivalent/multipeptide high molecular weight peptide molecules can be used to synthesize the peptide antigens. This will be achieved using known in the art and novel ligation strategies.

<u>Preparation of Synthetic Peptides</u>

Peptides from Table 1 can be synthesized in such away as to contain two ligands, which can be the same or different, which may or may not be the complementary ligand. These bi-modal peptides can incorporate any

As will be readily apparent to persons skilled in this area these peptides may be used as antigens in diagnostic tests or as immunogens in formulations.

In a third aspect the present invention consists in an antibody preparation comprising antibodies specifically directed against the composition of the first aspect of the invention or the peptides of the second aspect of the invention. The antibodies may be either polyclonal or monoclonal antibodies.

In a fourth aspect the present invention consists in a method of treating a subject suffering from *Porphyromonas gingivalis* infection, the method comprising administering to the subject an effective amount of the antibody preparation of the third aspect.

In a preferred embodiment the antibody preparation is administered as a mouth wash or as a dentifrice.

In a fifth aspect the present invention consists in a method of treating a subject suffering from *Porphyromonas gingivalis* infection, the method comprising administering to the subject an effective amount of a composition of the first aspect of the invention or a peptide of the second aspect of the invention.

In a preferred embodiment the composition or peptide is administered as a mouth wash or as a dentifrice.

In a sixth aspect the present invention consists in a method of reducing the prospect of *P. gingivalis* infection in an individual and/or severity of disease, the method comprising administering to the individual an amount of the composition of the first aspect effective to induce an immune response in the individual directed against *P. Gingivalis*.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Peptides can be synthesized using one of the several methods of peptide synthesis known in the art including standard solid phase peptide synthesis using t-butyloxycarbonyl amino acids (Mitchell et al., 1978, J. Org. Chem. 43:2845-2852) using 9-fluorenylmethyloxycarbonyl (Fmoc) amino acids on a polyamide support (Druland et al., 1986, J. Chem. Soc.Perkin

Trans. 1 125-137) by pepscan synthesis (Geysen et al., 1987, J. Immunol Methods 03:259; 1984, Proc. Natl. Acad. Sci. USA, 81:3998) or by standard liquid phase synthesis.

A variety of methods for the synthesis of multivalent/multipeptide high molecular weight peptide molecules can be used to synthesize the peptide antigens. This will be achieved using known in the art and novel ligation strategies.

Preparation of Synthetic Peptides

Peptides from Table 1 can be synthesized in such away as to contain two ligands, which can be the same or different, which may or may not be the complementary ligand. These bi-modal peptides can incorporate any ligand thus linkages such as thioether, thioester, hydrazone, oxime, thiazolidine can be utilised for the synthesis of multipeptide constructs Shao and Tam., 1995, J. Am. Chem. Soc. 117, 3893-3899, Rose, et al 1996, Bioconjugate Chem. 7(5):552-556, Rose. K., 1994, J. Am. Chem. Soc. 116:30-33, Canne., et al 1995, J. Am. Chem. Soc. 117:2998-3007, Lu., et al, 1991, Mol. Immunol 28(6):623-630, Liu and Tam., 1994, Proc. Natl. Acad. Sci. 91,:6584-6588. A novel ligating strategy is to use the known reaction between thioanisole and acryloyl peptides (O'Brien-Simpson et al., 1997, J. Am. Chem. Soc. 119 (6) which results in the para substitution of thioanisole by the double bond in acidic conditions. By synthesising and mixing acryloyl-peptides and phenylthio acetyl peptides and exposing them to acidic conditions ligation can proceed by Friedal-Craft alkylation. Ligation can be accomplished between peptides and on to an oligolysine support derivatised with one of the ligands. Conditions for ligation can consist of; Friedal-Craft reaction conditions which are known in the art and known peptide cleavage conditions.

The introduction of ligand groups to form bi-modal peptides can be achieved by coupling a ligand on to free amino groups, which is known in the art. at the N- or C- terminus of a peptide or within the peptide sequence. This can be achieved by coupling eg. Fmoc(Fmoc) 2,3 diamino propionic acid or Fmoc Lys (Fmoc)-OH or orthogonally protected lysine residues such as Fmoc Lys (Mtt)-OH using standard peptide coupling protocols on to the N-terminus or introduced at the C-terminus or within the peptide sequence. After deprotection, ligand groups can be coupled on to the amino groups and

by selective deprotection of eg. Fmoc Lys (Mtt) different ligands can be coupled on to a single peptide. At any point in the synthesis spacer moieties can be introduced between the peptide and the ligands and/or between the ligands, which may be used to reduce steric hindrance in the ligation reaction. Figure 1 shows the synthesis protocol.

Peptide ligation can be achieved in solution or on the solid phase. The incorporation of different ligands and selective protection of one ligand can allow the synthesis of multivalent, multipeptide constructs, where by, peptides are ligated sequentially. This strategy has the advantage that the orientation and order of peptides ligated is known and can be controlled. Protecting groups for ligands can be for example Fmoc, allyloxycarbonyl (Aloc) or nitrocinnamyloxycarbonyl (Noc) which are stable to standard cleavage conditions but are easily removed under basic conditions or catalytic allyl transfer. Figure 2 shows the ligation scheme for the synthesis of multivalent peptide constructs using bi-modal peptides. The protocol can be adapted for a variety of ligation chemistries by simply altering the ligands which are coupled to the peptide to form the bi-modal peptide.

The step wise addition of each peptide can be achieved on the solid phase. This can be achieved by synthesising a peptide on to the solid support via a base labile handle eg. 4-hydroxymethyl benzoic acid. This can allow full side chain deprotection of the peptide with the peptide remaining attached to the solid support. This would allow ligation to still be carried out in aqueous solvents similar to those used for solution phase ligation except that separation of the ligand product from unreacted bi-modal peptide can be achieved by simply washing the solid support. The reaction can be monitored by ninhydrin or trinitrobenzene sulphonic acid tests, where by, lysine residues within the bi-modal peptide would need to be protected eg. with (4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde) which is stable to acid cleavage but can be removed with hydrazine. Figure 3 shows the ligation strategy for the solid phase.

Bi-modal peptides can be synthesized so that ligands are at the N- and C- terminus. This would allow the preparation of cyclic peptides and the formation of di-peptide constructs where by peptides can run parallel or anti parallel to each other by either coupling N- to N- and C- to C- termini or N- to C- termini together respectively (Figure 4).

Another technique for the synthesis of multivalent peptide constructs is to ligate peptides on to an oligolysine support (Rose, et al 1996, Bioconjugate Chem. 7(5):552-556, Canne., et al 1995, J. Am. Chem. Soc. 117:2998-3007 and Lu., et al, 1991, Mol. Immunol 28(6):623-630). By incorporating a number of different ligands and or protected ligands on to the lysine support, peptides can be ligated to a particular position on the support. Ligation chemistries such as oxime or hydrazone with haloacylation and Friedal-Craft alkylation can be used sequentially without the need for ligand protection. Ligand protection can be used to increase the number of different peptides incorporated on to the lysine support. Figure 5 demonstrates the synthesis protocol.

Another method known in the art is the synthesis of acryloyl peptides and their polymerisation with acrylamide (O'Brien-Simpson et al., 1997, J. Am. Chem. Soc. 119 (6)) or acryloyl amino acids. Peptides from the PrtR-PrtK protein complex listed in Table 1 can be acryloylated and polymerised either singularly or in combination. Although this method allows the polymerisation of a number of peptides together the order in which peptides are incorporated can not be controlled.

The final peptide construct may or may not contain all, sum or part of the peptides listed in Table 1. Also the construct may or may not contain promiscuous T-cell epitopes known in the art (Kaumaya et al 1994, in Solid Phase Synthesis, Ed Epton, R) or a derived sequence from structural/binding motifs of MHC class II binding peptides (O'Sullivan et al., 1991, J. Immunol, 147:2663-2669, Hammer et al., 1993, Cell, 74:197-203 and Alexander et al., 1994, Immunity, 1:751-761). Furthermore, lipid moieties such as palmitic acid or cholesterol can be included to enhance the immunogenic properties of the peptide construct. Enzymatic cleavable sequences known in the art (Duncan et al., ref) or derived sequences from cleavage motifs (Van Noort and van der Drift., ref) can also be incorporated with the peptide construct.

The synthetic peptide antigens identified in Table 1 are of particular interest for diagnostics and neutralisation by passive immunity through oral compositions containing neutralising antibodies and by vaccine development. The superiority of these synthetic peptide antigens to prior disclosed *P. gingivalis* antigens, is that these sequences are homologous to structurally and functionally significant areas of the major *P. gingivalis* virulence factor the PrtR-PrtK proteinase-adhesin complex. The peptides

represent sequences associated with the active site of the proteinases and binding domains of the adhesins making them ideal for the development of diagnostic and immunoprophylactic products.

Antibodies against the antigens can be used in oral compositions such as toothpaste and mouthwash to neutralise the antigens and thus prevent disease. Antigen-specific antibodies can also be used for the early detection of *P. gingivalis* in subgingival plaque samples by a diagnostic assay. A vaccine based on these antigens and suitable adjuvant delivered by nasal spray, orally or by injection to produce a specific immune response against these antigens thereby reducing colonisation and virulence of *P. gingivalis* and thereby preventing disease. The peptide antigens of the present invention may be used as immunogens in prophylactic and/or therapeutic vaccine formulations; or as an antigen in diagnostic immunoassays directed to detection of *P. gingivalis* infection by measuring an increase in serum titer of *P. gingivalis* - specific antibody. Also the synthetic peptides of the present invention may be used to generate antigen-specific antibody which may be useful for passive immunization and as reagents for diagnostic assays directed to detecting the presence of *P. gingivalis* in clinical specimens such as subgingival plaque samples.

Unlike whole *P. gingivalis* cells or other previously prepared antigens, the synthetic peptide antigens described herein are safe and effective antigens for the preparation of a vaccine for the prevention of *P. gingivalis*-associated periodontal disease.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Synthesis of Bi-modal Peptides Although a specific example is shown here any ligand can be introduced at the a or e amino groups of lysine. (a) acylation e.g. amino acid:HOBt:HBTU:DIPEA 1:1:1:1.5 in dimethyl formamide (DMF). (b) Fmoc deprotection e.g. 20% piperidine in DMF. (c) Levulinic acid: diisopropyl carbodiimide (DIC) 2:1 in dichloromethane (DCM). 1h. (d) Mtt removal, 3x 1% TFA in DCM, 3 mins. (e) Fmoc-Hydrazino benzoic acid : DIC 2:1, in DCM, 1h. (f) Acid cleavage e.g. TFA : water 95:5.

Figure 2: Synthesis of multivalent peptide constructs using bi-modal peptides. (a) Ligation. 8 M urea and 0.1 M NaH_2PO_4 (pH range 3-4.7). Ligation can be monitored by reverse phase analytical HPLC and mass spectrometry. (b) Deprotection, e.g. Aloc is removed by palladium(0)-catalyzed allyl gropu transfer to a basic receptor. The ligation product can be purified by preparative HPLC and lypholised. (c) Ligation. Similar conditions as described in (a). Different ligation chemistries can be used by synthesising peptides with different ligands and synthesising non-complementary ligands on to the same peptide, thereby avoiding proected ligands. The square symbol indicates protection, (L) ligand, (P) peptide.

Figure 3: Synthesis of multivalent peptide constructs using bi-modal peptides by solid phase. (a) Deprotection and ligation. The S-acetyl protecting group is removed by aqueous hydroxyamine 0.05 M, pH 7.3. After washing the first peptide can be ligated on to the SH group, 6 M aqueous guanidine hydrochloride and 0.05 M EDTA pH 6.4-6.5 adjusted by 1 M Tris.HCl under nitrogen. Ligation buffer can contain organic solvents such as acetonitrile. (b) Deprotection, the S-acetyl protecting group can be removed by aqueous hydroxyamine 0.05 M, pH 7.3. (c) Ligation, as described in (a) although different ligation chemistries can be used by synthesising peptides with different ligands and synthesising non-complementary ligands on to the same peptide, thereby avoiding proected ligands. The square symbol indicates protection, (L) ligand, (P) peptide, (B) base labile handle, 4-hydroxymethyl benzoic acid.

Figure 4: Cyclization using bi-modal peptides. (a) Deprotection and cyclisation. Synthesis of bi-modal peptides which have complimentary ligands at their N- and C- termini allows the cyclisation of these peptides in aqueoous buffers. (i) Ligation. (ii) Deprotection and ligaction. (iii) Cleavage of the cyclic peptide from the base labile handle. Example: The peptides shown are from Table 1 and present the active site peptides from prtR 45. (a) Ligation. 95% aqueous TFA. . Ligation can be monitored by reverse phase analytical HPLC and mass spectrometry. Ligation conditions can be varied to included scavangers commonly used in peptide synthesis and different acidic conditions to enhance the Friedal-Craft alkylation. (b) Deprotection and ligation. The S-acetyl protecting group can removed by aqueous

hydroxyamine 0.05 M, pH 7.3. Ligation, 6 M aqueous guanidine hydrochloride and 0.05 M EDTA pH 6.4-6.5 adjusted by 1 M Tris.HCl under nitrogen. The ligation straegy can also be accomplished on the solid phase. By selecting which ligand to introduce at the N- and C- terminal parallel and anti-parallel cyclic peptides can be synthesised.

Figure 5: Synthesis of multivalent multiple antigenic peptides (MAPs) using alternate ligation chemistries. By using different ligation strategies a vareity of peptides can be ligated onto a single multiple antigenic peptide. The example shown is of peptides listed in Table 1. (a) Ligation, 95% aqueous TFA. . Ligation can be monitored by reverse phase analytical HPLC and mass spectrometry. Deprotection, Aloc can removed by palladium(0)-catalyzed allyl group transfer to a basic receptor. after purifaction the second peptide can be ligated on to the MAP, (c) 8 M urea and 0.1 M NaH_2PO_4 (pH range 3-4.7).

Figure. 6 Gel Filtration FPLC of pooled and concentrated fractions eluting from Q sepharose anion exchange FPLC. Anion exchange fractions eluting between 160-246 mM NaCl and representing the leading edge of the main peak of proteolytic/amidolytic activity were pooled, equilibrated in TC buffer pH 7.4 containing 50 mM NaCl, concentrated and applied to Superose 12 HR 10/30 gel filtration column using the same buffer at a flow rate of 0.3 ml min^{-1}. Fractions (0.5 ml) were assayed for proteolytic/amidolytic activity using azocasein, Bz-L-Arg-*p*NA and z-L-Lys-*p*NA. Amidolytic activity of each 0.5 ml fraction with Bz-L-*p*NA is shown by the histogram.

Figure. 7 SDS-PAGE (bolied/reduced conditions) of the anion exchange (Mono Q) peak eluting at 200 mM NaCl and containing only Arg-specific activity. Lane 1, Pharmacia low molecular mass standards; lane 2, Purified 50 kDa Arg-specific proteinase, PrtRII50.

Figure. 8 An alignment of the deduced amino acid sequences of PrtRII50, PrtR45 Arg-specific proteinase and PrtK48 Lys-specific proteinase with optimised similarity. The amino acyl residues of PrtRII50 are numbered from the N-terminal residue of the mature protein. * indicates an identical residue

Figure. 10 Competitive Binding Assay demonstrating binding of the TLCK-inactivated PrtR-PrtK proteinase-adhesion complex to the synthetic peptide corresponding to the putative adhesin binding motif (ABM). -●-●- ABM synthetic peptide. PYQPVSNLTATTQGQKVTLKWDAPSTK. -■-■- Control peptide, FNGGISLANYTGHGSETAWGT corresponding to residues 428-448 of PrtR45. -▲-▲- casein. See Materials and Methods for details.

Figure 11. Average lesion size of mice challenged with Porphyromonas gingivalis in a mouse abcsess model. BALB/c mice (6 per group) were inoculated (s.c.) with 50μg of antigen emulsified in CFA and IFA for the primary and secondary inoculations and then challenged (s.c.) with 8 X 10^9 cells of P. gingivalis strain 33277. ABM1(R45)-DT, (□); ABM2(K39)-KT, (○); ABM3(R44)-DT, (✱); ABM4(R17)-DT, (●); ABM5(R15)-DT, (◆); ABM6(K39)-DT, (◇); PAS1(R45)-DT, (▲); PAS1(K48)-DT, (■); Control peptide-DT, (-◇-); formalin killed P. gingivalis strain 33277, (+); DT, (- -△- -); adjuvant, (×). For clarity error bars are not shown.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to an oral composition and an immunogenic composition for the suppression of the pathogenic effects of the intra-oral bacterium *Porphyromonas gingivalis* associated with periodontal disease. It also relates to diagnostic tests for the presence of *Porphyromonas gingivalis* in subgingival plaque samples and specific anti-*P. gingivalis* antibodies in sera. The peptide antigens of Table 1 can be synthesized individually or as multimetric or multipeptide constructs.

The synthetic peptide antigens are used to generate polyclonal or monoclonal antibodies using standard techniques. The animals used for antibody generation can be mice, rabbits, goats, chickens, sheep, horses, cows etc. When a high antibody titre against the antigens is detected by immunoassay the animals are bled or eggs or milk are collected and the serum prepared and/or antibody purified using standard techniques or monoclonal antibodies produced by fusing spleen cells with myeloma cells using standard techniques. The antibody (immunoglobulin fraction) may be

This invention relates to an oral composition and an immunogenic composition for the suppression of the pathogenic effects of the intra-oral bacterium *Porphyromonas gingivalis* associated with periodontal disease. It also relates to diagnostic tests for the presence of *Porphyromonas gingivalis* in subgingival plaque samples and specific anti-*P. gingivalis* antibodies in sera. The peptide antigens of Table 1 can be synthesized individually or as multimetric or multipeptide constructs.

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For oral compositions it is preferred that the amount of the above antibodies administered is 0.0001 -50 g/kg/day and that the content of the above antibodies is 0.0002 - 10% by weight preferably 0.002 -5% by weight of the composition. The oral composition of this invention which contains the

above-mentioned serum or milk antibody may be prepared and used in various forms applicable to the mouth such as dentifrice including toothpastes, toothpowders and liquid dentifrices, mouthwashes, troches, chewing gums, dental pastes, gingival massage creams, gargle tablets, dairy products and other foodstuffs. The oral composition according to this invention may further include additional well known ingredients depending on the type and form of a particular oral composition.

In certain highly preferred forms of the invention the oral composition may be substantially liquid in character, such as a mouthwash or rinse. In such a preparation the vehicle is typically a water-alcohol mixture desirably including a humectant as described below. Generally, the weight ratio of water to alcohol is in the range of from about 1:1 to about 20:1. The total amount of water-alcohol mixture in this type of preparation is typically in the range of from about 70 to about 99.9% by weight of the preparation. The alcohol is typically ethanol or isopropanol. Ethanol is preferred.

The pH of such liquid and other preparations of the invention is generally in the range of from about 4.5 to about 9 and typically from about 5.5 to 8. The pH is preferably in the range of from about 6 to about 8.0, preferably 7.4. The pH can be controlled with acid (e.g. citric acid or benzoic acid) or base (e.g. sodium hydroxide) or buffered (as with sodium citrate, benzoate, carbonate, or bicarbonate, disodium hydrogen phosphate, sodium dihydrogen phosphate, etc).

Other desirable forms of this invention, the oral composition may be substantially solid or pasty in character, such as toothpowder, a dental tablet or a dentifrice, that is a toothpaste (dental cream) or gel dentifrice. The vehicle of such solid or pasty oral preparations generally contains dentally acceptable polishing material. Examples of polishing materials are water-insoluable sodium metaphosphate, potassium metaphosphate, tricalcium phosphate, dihydrated calcium phosphate, anhydrous dicalcium phosphate, calcium pyrophosphate, magnesium orthophosphate, trimagnesium phosphate, calcium carbonate, hydrated alumina, calcined alumina, aluminum silicate. zirconium silicate, silica, bentonite, and mixtures thereof. Other suitable polishing material include the particulate thermosetting resins such as melamine-, phenolic, and urea-formaldehydes, and cross-linked polyepoxides and polyesters. Preferred polishing materials include crystalline

silica having particle sized of up to about 5 microns, a mean particle size of up to about 1.1 microns, and a surface area of up to about 50,000 $cm^2/gm.$, silica gel or colloidal silica, and complex amorphous alkali metal aluminosilicate.

When visually clear gels are employed, a polishing agent of colloidal silica, such as those sold under the trademark SYLOID as Syloid 72 and Syloid 74 or under the trademark SANTOCEL as Santocel 100, alkali metal alumino-silicate complexes are particularly useful since they have refractive indices close to the refractive indices of gelling agent-liquid (including water and/or humectant) systems commonly used in dentifrices.

Many of the so-called "water insoluble" polishing materials are anionic in character and also include small amounts of soluble material. Thus, insoluble sodium metaphosphate may be formed in any suitable manner as illustrated by Thorpe's Dictionary of Applied Chemistry, Volume 9, 4th Edition, pp. 510-511. The forms of insoluble sodium metaphosphate known as Madrell's salt and Kurrol's salt are further examples of suitable materials. These metaphosphate salts exhibit only a minute solubility in water, and therefore are commonly referred to as insoluble metaphosphates (IMP). There is present therein a minor amount of soluble phosphate material as impurities, usually a few percent such as up to 4% by weight. The amount of soluble phosphate material, which is believed to include a soluble sodium trimetaphosphate in the case of insoluble metaphosphate, may be reduced or eliminated by washing with water if desired. The insoluble alkali metal metaphosphate is typically employed in powder form of a particle size such that no more than 1% of the material is larger than 37 microns.

The polishing material is generally present in the solid or pasty compositions in weight concentrations of about 10% to about 99%. Preferably, it is present in amounts from about 10% to about 75% in toothpaste, and from about 70% to about 99% in toothpowder. In toothpastes, when the polishing material is silicious in nature, it is generally present in amount of about 10-30% by weight. Other polishing materials are typically present in amount of about 30-75% by weight.

In a toothpaste, the liquid vehicle may comprise water and humectant typically in an amount ranging from about 10% to about 80% by weight of the preparation. Glycerine, propylene glycol, sorbitol and polypropylene glycol exemplify suitable humectants/carriers. Also

advantageous are liquid mixtures of water, glycerine and sorbitol. In clear gels where the refractive index is an important consideration, about 2.5 - 30% w/w of water, 0 to about 70% w/w of glycerine and about 20-80% w/w of sorbitol are preferably employed.

Toothpaste, creams and gels typically contain a natural or synthetic thickener or gelling agent in proportions of about 0.1 to about 10, preferably about 0.5 to about 5% w/w. A suitable thickener is synthetic hectorite, a synthetic colloidal magnesium alkali metal silicate complex clay available for example as Laponite (e.g. CP, SP 2002, D) marketed by Laporte Industries Limited. Laponite D is, approximately by weight 58.00% SiO_2, 25.40% MgO, 3.05% Na_2O, 0.98% Li_2O, and some water and trace metals. Its true specific gravity is 2.53 and it has an apparent bulk density of 1.0 g/ml at 8% moisture.

Other suitable thickeners include Irish moss, iota carrageenan, gum tragacanth, starch, polyvinylpyrrolidone, hydroxyethylpropylcellulose, hydroxybutyl methyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose (e.g. available as Natrosol), sodium carboxymethyl cellulose, and colloidal silica such as finely ground Syloid (e.g. 244). Solubilizing agents may also be included such as humectant polyols such propylene glycol, dipropylene glycol and hexylene glycol, cellosolves such as methyl cellosolve and ethyl cellosolve, vegetable oils and waxes containing at least about 12 carbons in a straight chain such as olive oil, castor oil and petrolatum and esters such as amyl acetate, ethyl acetate and benzyl benzoate.

It will be understood that, as is conventional, the oral preparations are to be sold or otherwise distributed in suitable labelled packages. Thus, a jar of mouthrinse will have a label describing it, in substance, as a mouthrinse or mouthwash and having directions for its use; and a toothpaste, cream or gel will usually be in a collapsible tube, typically aluminium, lined lead or plastic, or other squeeze, pump or pressurized dispenser for metering out the contents, having a label describing it, in substance, as a toothpaste, gel or dental cream.

Organic surface-active agents are used in the compositions of the present invention to achieve increased prophylactic action. assist in achieving thorough and complete dispersion of the active agent throughout the oral cavity, and render the instant compositions more cosmetically acceptable. The organic surface-active material is preferably anionic,

nonionic or ampholytic in nature which does not denature the antibody of the invention, and it is preferred to employ as the surface-active agent a detersive material which imparts to the composition detersive and foaming properties while not denaturing the antibody. Suitable examples of anionic surfactants are water-soluble salts of higher fatty acid monoglyceride monosulfates, such as the sodium salt of the monosulfated monoglyceride of hydrogenated coconut oil fatty acids, higher alkyl sulfates such as sodium lauryl sulfate, alkyl aryl sulfonates such as sodium dodecyl benzene sulfonate, higher alkylsulfo-acetates, higher fatty acid esters of 1,2-dihydroxy propane sulfonate, and the substantially saturated higher aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds, such as those having 12 to 16 carbons in the fatty acid, alkyl or acyl radicals, and the like. Examples of the last mentioned amides are N-lauroyl sarcosine, and the sodium, potassium, and ethanolamine salts of N-lauroyl, N-myristoyl, or N-palmitoyl sarcosine which should be substantially free from soap or similar higher fatty acid material. The use of these sarconite compounds in the oral compositions of the present invention is particularly advantageous since these materials exhibit a prolonged marked effect in the inhibition of acid formation in the oral cavity due to carbohydrates breakdown in addition to exerting some reduction in the solubility of tooth enamel in acid solutions. Examples of water-soluble nonionic surfactants suitable for use with antibodies are condensation products of ethylene oxide with various reactive hydrogen-containing compounds reactive therewith having long hydrophobic chains (e.g. aliphatic chains of about 12 to 20 carbon atoms), which condensation products ("ethoxamers") contain hydrophilic polyoxyethylene moieties, such as condensation products of poly (ethylene oxide) with fatty acids, fatty alcohols, fatty amides, polyhydric alcohols (e.g. sorbitan monostearate) and polypropyleneoxide (e.g. Pluronic materials).

Surface active agent is typically present in amount of about 0.1-5% by weight. It is noteworthy, that the surface active agent may assist in the dissolving of the antibody of the invention and thereby diminish the amount of solubilizing humectant needed.

Various other materials may be incorporated in the oral preparations of this invention such as whitening agents, preservatives, silicones, chlorophyll compounds and/or ammoniated material such as urea, diammonium phosphate, and mixtures thereof. These adjuvants, where

present, are incorporated in the preparations in amounts which do not substantially adversely affect the properties and characteristics desired.

Any suitable flavoring or sweetening material may also be employed. Examples of suitable flavoring constituents are flavoring oils, e.g. oil of spearmint, peppermint, wintergreen, sassafras, clove, sage, eucalyptus, marjoram, cinnamon, lemon, and orange, and methyl salicylate. Suitable sweetening agents include sucrose, lactose, maltose, sorbitol, xylitol, sodium cyclamate, perillartine, AMP (aspartyl phenyl alanine, methyl ester), saccharine, and the like. Suitably, flavor and sweetening agents may each or together comprise from about 0.1% to 5% more of the preparation.

In the preferred practice of this invention an oral composition according to this invention such as mouthwash or dentifrice containing the composition of the present invention is preferably applied regularly to the gums and teeth, such as every day or every second or third day or preferably from 1 to 3 times daily, at a pH of about 4.5 to about 9, generally about 5.5 to about 8, preferably about 6 to 8, for at least 2 weeks up to 8 weeks or more up to a lifetime.

The compositions of this invention can be incorporated in lozenges, or in chewing gum or other products, e.g. by stirring into a warm gum base or coating the outer surface of a gum base, illustrative of which may be mentioned jelutong, rubber latex, vinylite resins, etc., desirably with conventional plasticizers or softeners, sugar or other sweeteners or such as glucose, sorbitol and the like.

Another important form of the invention is a immunogenic composition based on the synthetic peptide antigens and suitable adjuvant delivered by nasal spray, orally or by injection to produce a specific immune response against the antigen thereby reducing colonisation of *P. gingivalis* and reducing virulence thereby preventing disease. Unlike whole *P. gingivalis* cells or other previously prepared antigens, the peptide antigens described herin are safe and effective antigens for the preparation of a vaccine for the prevention of *P. gingivalis*-associated periodontal disease. Additionally, according to the present invention, antigenic peptide produced may be used to generate *P. gingivalis* antisera useful for passive immunization against periodontal disease and infections caused by *P. gingivalis*.

The following examples are further illustrative of the nature of the present invention, but it is understood that the invention is not limited thereto. All amounts and proportions referred to herein and in the appended claims are by weight unless otherwise indicated.

EXAMPLE 1

The identification of the proteinase active site and adhesin binding motifs was facilitated by the cloning and characterisation of the second gene encoding an Arg-specific proteinase of *P. gingivalis* W50.

Materials

O-Benzotriazole-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), 1hydroxybenzotriazole (HOBt), diisopropylethylamine (DIPEA), *N,N*-dimethylformamide (DMF), piperidine, trifluoroacetic acid (TFA) and 9-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids were obtained from Auspep Pty Ltd (Melbourne, Australia). Triisopropylsilane (TIPS) and ethanedithiol (EDT) were obtained from Aldrich (New South Wales, Australia). 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was obtained from Sigma Chemical Company (New South Wales, Australia). Phenol and diethyl ether were obtained from BDH (Poole, UK). Unless otherwise stated chemicals were of peptide synthesis grade or its equivalent.

Bacterial strain and growth conditions

Lyophilized cultures of *Porphyromonas gingivalis* W50 were kindly provided by Professor P. Marsh (PHLS, Centre for Applied Microbiology and Research, Wiltshire, UK). *P. gingivalis* W50 was grown anaerobically (Bhogal *et al.*, 1997) and *Escherichia coli* JM109 and LE392 strains were grown following the procedures previously described (Slakeski *et al.*, 1996).

Purification of the 50 kDa Arg-specific proteinase.

P. gingivalis W50 was grown in batch culture (5 L) and harvested at late logarithmic phase by centrifugation (5,000 x *g*, 20 min, 4 °C). Cells were washed once with 150 ml TC buffer (20 mM Tris-HCl pH 7.4 and 5 mM $CaCl_2$) containing 50 mM NaCl and sonicated as described previously (Bhogal

et al., 1997). The sonicate was centrifuged (100,000 x *g*, 30 min, 4 °C) and the supernatant filtered (0.22 μm) prior to anion-exchange FPLC. The sonicate was applied to an anion-exchange column (Hiload XK 16/10 Q Sepharose, Pharmacia-LKB) cooled to 4 °C, in multiple injections using a 50 ml superloop (Pharmacia-LKB). The sonicate was eluted using a linear gradient from 0-100% buffer B over 90 min at a flow rate of 2.0 ml min^{-1}. Absorbance was monitored at 280 nm and elutant collected at 4 °C in 6 ml fractions using a Frac 100 fraction collector (Pharmacia-LKB). Buffer A was TC buffer containing 50 mM NaCl and buffer B was TC buffer containing 500 mM NaCl. Fractions were analysed for proteolytic and amidolytic activity using azocasein (A-2765, Sigma Chemical Co. St Louis, MO), benzoyl-L-Arg-*p*-nitroanilide (Bz-L-Arg-*p*NA, Sigma) and benzyloxycarbonyl-L-Lys-*p*-nitroanilide (z-L-Lys-*p*NA, Calbiochem, Melbourne, Australia) as described previously (Bhogal *et al.*, 1997) except that fractions were pre-incubated with 10 mM cysteine for 10 min at 25 °C before the addition of substrate. For the amidolytic assays absorbance was monitored at 410 nm as previously described (Bhogal *et al.*, 1997) and the amidolytic activity expressed as U where U = μmol substrate converted min^{-1} at 25 °C.. Anion-exchange fractions eluting between 160-246 mM NaCl containing the highest ratio of Arg-specific to Lys-specific activity were, washed and concentrated in TC buffer containing 150 mM NaCl using a centripep and centricon 10 concentrators (Amicon) and applied to a gel filtration column (Superose 12, HR 10/30, Pharmacia-LKB) using TC buffer containing 150 mM NaCl at a flow rate of 0.3 ml min^{-1}. Absorbance was monitored at 280 nm and fractions collected at 4 °C using a Frac 100 fraction collector. The M_r values of eluant peaks were determined using gel filtration molecular mass standards (Pharmacia-LKB). The peak eluting at 50 kDa containing only Arg-specific amidolytic activity was washed in TC buffer containing 50 mM NaCl using a centricon-10 concentrator (Amicon) and applied to a Mono Q (HR 5/5) anion-exchange column using a 5 ml loop and eluted using a linear gradient of 0-100% buffer B at a flow rate 1.0 ml min^{-1}. Buffer A was TC buffer containing 150 mM NaCl, buffer B was TC buffer containing 500 mM NaCl. Absorbance was monitored at 280 nm and fractions collected at 4 °C using a Frac 100 fraction collector.

<u>SDS-PAGE, protein transblot and *N*-terminal sequence analysis</u>

SDS-PAGE was performed using a Mini protean II electrophoresis system (Biorad) with 12% (w/v), 1 mm separating gels, overlaid with 5% stacking gels (Laemmli, 1970) and proteins transblotted and N-terminally sequenced using the procedures previously described (Bhogal *et al.*, 1997).

Cloning and nucleotide sequence analysis

The *P. gingivalis* W50 LambdaGEM®-12 genomic library, described previously (Slakeski *et al.*, 1996) was screened using synthetic oligonucleotides derived from the nucleotide sequence of *prtR* (Slakeski *et al.*, 1996) corresponding to the N-terminal sequence of PrtR45. Oligonucleotide probes were 5' end-labelled using γ^{32}P ATP and T4 polynucleotide kinase. Approximately 1.5 x 10^4 phage were screened by lifting onto Nylon membrane filters and hybridising with radiolabelled oligonucleotides overnight in hybridisation buffer: 6 x SSC (SSC is 15 mM sodium citrate, 150 mM NaCl pH 8.0), 0.25% SDS, 5 x Denhardt's solution (Sambrook *et al.*. 1989) and 100 μg/ml salmon sperm DNA at 49 °C. Filters were washed extensively in a solution of 2 x SSC containing 0.1% SDS (w/v) at 49 °C. Phage from positively-hybridising plaques were purified using standard procedures (Sambrook *et al.*, 1989). Phage DNA was digested with *Eco*72 I and the resulting fragments ligated into *Sma* I-BAP pUC18 (Pharmacia, Sydney, Australia) which was used to transform *E. coli* JM109 using the heat shock procedure (Sambrook *et al.*, 1989). Double-stranded template DNA was sequenced as described previously (Slakeski *et al.*, 1996).

PCR was used to amplify a 991 bp fragment containing the internal *Eco* 72I site encoded by *prtRII* using the two oligonucleotide primers 5'-CGGCTTCCGTAAAGTC-3' (forward primer identical to bases 657-672 of *PrtRII* sequence) and 5'-TGGCTACGATGACGATCATACGAC-3' (reverse primer with 96% identity to bases 1624-1647 of *PrtRII*). The PCR was carried out in a final volume of 100 μl and each reaction mixture contained 100 ng *P. gingivalis* W50 genomic DNA, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 100 pmol of each primer, 20 mM Tris-HCl, pH 8.4, 50 mM KCl and 2.5 U Taq DNA Polymerase (Gibco BRL). The reaction mixture was heated at 95 °C for 3 min and then subject to 25 cycles of DNA denaturation at 95 °C for 30 s, primer annealing at 40 °C for 1 min and extension at 72 °C for 2 min. Following cycling, the reaction mixture was finally heated at 72 °C for 5 min.

Amplified DNA was purified using a PCR Spinclean Kit (Progen) and sequenced across the *Eco* 72I site in both directions.

Purification of high molecular mass complexes of Arg-specific and Lys-specific proteinases and adhesins (PrtR-PrtK complexes)

The high molecular mass, cell-associated proteinase-adhesin complexes (PrtR-PrtK complexes) of *P. gingivalis* W50 were purified using a combination of anion-exchange, gel filtration and Arg-sepharose affinity chromatography from a cell sonicate as described previously (Bhogal *et al.*, 1997). The complexes were characterised using SDS-PAGE, transblotting and sequence analysis and assayed for enzymic activity using Bz-L-Arg-*p*NA and Z-L-Lys-*p*NA substrates (Bhogal *et al.*, 1997).

Solid-phase peptide synthesis

Peptides were synthesised manually using standard Fmoc solid-phase peptide synthesis protocols. The peptides were assembled as the carboxyamide form using Fmoc-Pal-Peg-PS resin (PerSeptive Biosystems Inc., Framingham, MA). Coupling was accomplished with HBTU/HOBt activation using 4 equiv of Fmoc-amino acid and 6 equiv of DIPEA. The Fmoc group was removed by 2% v/v DBU in DMF containing 2% v/v piperidine. Cleavage of peptides from the resin support was performed using TFA:phenol:TIPS:EDT:water (92:2:2:2:2) cleavage cocktail for 2.5 hours. After cleavage the resin was removed by filtration and the filtrate concentrated to approximately 1 ml under a stream of nitrogen. After the peptide products were precipitated in cold ether, they were centrifuged and washed three times. The peptide precipitate was then dissolved in 10 ml of water containing 0.1% v/v TFA and insoluble residue removed by centrifugation.

Purification of synthesized peptides was performed using a Brownlee C18 Aquapore ODS column (250 x 100 mm) installed in a Waters HPLC system. Chromatograms were developed at a flow rate of 5.0 ml min^{-1} using 0.1% v/v TFA in water (solvent A) and 0.1% v/v TFA in 90% aqueous acetonitrile (solvent B). Peptides were eluted with a gradient of 10-30% solvent B over 40 min. Analytical HPLC was carried out using a Brownlee C8 Aquapore RP-300 column (220 x 4.6 mm) installed in a Applied Biosytems HPLC system. Chromatograms were developed using solvent A and solvent B

at a flow rate of 1.0 ml min^{-1} and a 0-100% linear gradient of solvent B over 30 min. Material eluted from the columns was monitored by absorbance at 214 nm. Peptides were analysed by mass spectrometry using a PerSeptive Biosystems Voyager DE MALDI-TOF.

Competitive binding assay

Wells of flat-bottomed polyvinyl microtitre plates (Microtitre, Dynatech Laboratories, VA) were coated overnight at 4 °C using a solution (5 mg/ml) of the adhesin binding motif (ABM) peptide in 0.1M phosphate buffered saline, pH 7.4, containing 0.1% v/v Tween 20 (PBST) and 0.1%w/v sodium azide. After removal of the coating solution, 2% w/v skim milk powder in PBST was added to block the remaining uncoated plastic for 1 hour at room temperature and then washed (4 x PBST). A solution (1 mg/ml) of the PrtR-PrtK proteinase-adhesion complex (inactivated with 1mM TLCK) was incubated with known concentrations of ABM peptide, control peptide and casein for 1 hour and then transferred to the microtitre plates coated with the ABM peptide. Following incubation for 2 hours at 37 °C the plates were washed (5 x PBST). A 1/10,000 dilution of rabbit anti-PrtR-PrtK antisera in PBST containing 1% w/v skim milk powder was then added to the washed wells and incubated for 2 hours at 37 °C. Bound antibody was detected by incubation with horseradish peroxidase-conjugated goat immunoglobulin (Ig) directed against rabbit Ig (BioRad, Richmond, CA) for 1.5 hours at 37 °C. After washing (5 x PBST), substrate (0.4mM 3,3',5,5'-tetramethylbenzidine in 0.1M sodium acetate/citric acid buffer containing 0.004% v/v hydrogen peroxide) was added and colour development was stopped by addition of 2M H_2SO_4. Optical density (O.D.) at 450 nm was measured using a BioRad microplate reader model 450.

RESULTS

PrtRII50 Arg-specific proteinase purification and characterisation

The *P. gingivalis* W50 cell sonicate contained 0.36 mg ml^{-1} protein and 2.4 and 1.1 μmol min^{-1} mg $protein^{-1}$ activity with 1.0 mM Bz-L-Arg-*p*NA and z-L-Lys-*p*NA as substrates respectively at 25 °C. The sonicate was subjected to Q-sepharose anion exchange FPLC and proteolytic/amidolytic activity eluting between 160-246 mM NaCl was collected and concentrated using a centripep and centricon-10 concentrator (Amicon, Sydney, Australia).

This fraction represented the leading edge of the main peak of proteolytic/amidolytic activity and contained the highest ratio of Arg-specific activity to Lys-specific activity. After concentration, the fraction was applied to a Superose 12 gel filtration column (Fig. 6). Arg- and Lys- specific activity was associated with the high molecular mass eluting material corresponding to peaks with M_r values of 0.6 - 2.0 x 10^6 Da and 300 kDa as reported previously (Bhogal *et al.*, 1997). However, a lower molecular mass peak of 50 kDa was also observed, which displayed only Arg-specific activity and this peak was collected for further purification. The 50 kDa gel filtration peak was applied to a Mono Q anion exchange column and upon application of a NaCl gradient the Arg-specific activity eluted in a distinct peak at a NaCl concentration of 200 mM NaCl with a 28-fold purification over the original crude sonicate. The peak containing Arg-specific activity was subjected to SDS-PAGE which confirmed a single 50 kDa protein band (Fig. 7). The 50 kDa band was transblotted and subjected to N-terminal sequence analysis which provided the amino acyl sequence YTPVEEKENGRMIVIVPKKYEEDIED. The specificity of the 50 kDa proteinase for arginyl residues was confirmed by the enzyme cleaving Bz-L-Arg-*p*NA but not z-L-Lys-*p*NA. The Arg-specific 50 kDa enzyme was activated by thiols (particularly cysteine), not inhibited by the serine proteinase inhibitors, phenylmethyl sulfonyl fluoride or 4-(2-aminoethyl)-benzenesulfonyl fluoride but inhibited by sulphydryl-directed reagents, leupeptin and EDTA at similar concentrations to that which inhibited the PrtR45 (Bhogal *et al.*, 1997). Inhibition with EDTA could be reversed by the addition of excess Ca^{2+} and the pH optimum of the enzyme was 8.0 with minimal activity below pH 6.0.

Molecular cloning and sequence analysis of the *prtRII* gene

Screening of the *P. gingivalis* genomic library using oligonucleotide probes specific for the N-terminus of PrtR45 identified several positive clones. The DNA from these clones was extracted and subjected to Southern analysis to identify those containing the 12 kb *Bam*H I fragment previously proposed to correspond to the gene encoding the second Arg-specific proteinase (Slakeski *et al.*, 1996). Lambda clone 18, containing a 12 kb *Bam*H I fragment was chosen for further analysis and DNA was isolated from this clone and digested with *Eco*72 I and randomly cloned into plasmid *Sma* I-

BAP pUC 18. Adjacent 3.3 and 1.2 kb *Eco*72 I genomic fragments were sequenced in both directions to generate the entire *prtRII* nucleotide sequence (Genebank Accession No. AF007124). A 991 bp PCR fragment was generated and sequenced to confirm the sequence encompassing the internal *Eco*72 I site.

The *prtRII* ORF comprises 2208 bp (736 a.a. residues) and encodes a preproprotein consisting of a putative leader sequence and a profragment followed by the mature Arg-specific proteinase (507 a.a. residues) containing the exact N-terminal amino acyl sequence obtained for the purified 50 kDa enzyme (PrtRII50). The N-terminal sequence of the mature protein, like PrtR45, is immediately preceded by an Arg residue in the profragment.

The *prtRII* gene exhibits a high degree of similarity with the 5' two fifths of the *prtR* gene which encodes PrtR45 and its associated adhesins (Figs. 8 & 9). A comparison of the two translated sequences shows an overall similarity of 76% and 80% for the translated preprofragment and the proteinase domain, respectively. The *prtRII* however, does not encode any of the C-terminal haemagglutinin/adhesin domains encoded by the *prtR* and *prtK* genes being consistent with the finding that the purified PrtRII50 proteinase was not associated with adhesins. The M_r of the PrtRII50 mature proteinase deduced from the translated *prtRII* gene sequence is 55.6 kDa which is consistent with the 50 kDa obtained by SDS-PAGE (Fig. 7) and is slightly larger than the deduced M_r of 53.9 kDa for PrtR45 (Bhogal *et al.*, 1997).

The sequence alignment of the deduced amino acyl sequence of PrtRII50 with the PrtR45 Arg-specific proteinase and the PrtK48 Lys-specific proteinase (Slakeski *et al.*, 1996; Bhogal *et al.*, 1997) shows that PrtRII50 displays high sequence similarity (97.5% identity) to the adhesin-associated PrtR45 proteinase except for the C-terminal 80 amino acyl residues (Fig. 8). In fact, this C-terminal 80 residue sequence of PrtRII50 is similar (47% identity) to the C-terminal 80 residues of the PrtR27 adhesin domain, the last domain of the PrtR (Fig. 9). In contrast to the high sequence identity of the PrtRII50 and PrtR45 proteinases, there is lower overall similarity (25% identity) between the two adhesin-associated PrtR45 and PrtK48 proteinases except around the C-terminal region where the motif - GEPNPYQPVSNLTATTQGQKVTLKWDAPSTK- (underlined in Fig. 8) is almost identical in both proteinases but is absent in PrtRII50. Similar motifs

also occur in the PrtR44, PrtR17, PrtK39 and PrtK44 adhesin domains of PrtR and PrtK (Table 1 ABM1 peptides), which have led us to propose that this motif is an adhesin-binding motif involved in the association of the PrtR and PrtK proteinases and adhesins into large complexes.

Binding of the PrtR-PrtK complex to a synthethic peptide corresponding to a putative adhesin binding motif

A peptide (ABM1 [R45]) corresponding to the proposed adhesin binding motif PYQPVSNLTATTQGQKVTLKWDAPSTK, was synthesised and used to measure binding of the PrtR-PrtK complex. Specific binding of TLCK-inactivated PrtR-PrtK complex to the ABM peptide was demonstrated in a competitive binding assay where a 5-100 fold molar excess of the ABM peptide in solution was required to inhibit binding of the complex to the ABM peptide adsorbed onto the microtitre plate (Fig. 10). A control peptide, FNGGISLANYTGHGSETAWGT corresponding to residues 428-448 of PrtR45, as well as casein did not inhibit the binding of the TLCK-inactivated PrtR-PrtK complex to the adsorbed ABM peptide. The anti-PrtR-PrtK antisera did not bind to the ABM peptide in the absence of the PrtR-PrtK complex. The inactivation with TLCK ensured that the complex was not binding to the peptide through the active sites of the proteinases. This was also confirmed by lack of binding of the PrtR-PrtK complex to casein and a non-specific peptide of similar size and lysine content to the ABM peptide but of unrelated sequence. These results demonstrating specific binding of the TLCK-inactivated PrtR-PrtK complex to the ABM peptide therefore are consistent with the proposed role of this conserved motif in the association of the PrtR and PrtK proteinases and adhesins into large complexes.

DISCUSSION

Using a *P. gingivalis* W50 cell sonicate we have purified and characterised a second cell-associated, Arg-specific, calcium-stabilized cysteine proteinase that is almost identical to the previously characterised Arg-specific cysteine proteinase PrtR45 (Bhogal *et al.*, 1997). However, despite the almost identical enzymic characteristics and inhibitor/activator profile to PrtR45 the second enzyme exhibits a number of key differences. Firstly, the second enzyme designated PrtRII50, is a discrete enzyme not associated with adhesins. The Arg-specific cysteine proteinase, PrtR45, is a

45 kDa component of a large multi-protein complex of Arg- and Lys-specific proteinases and adhesins (Bhogal *et al.*, 1997). Secondly, PrtRII50 is slightly larger than PrtR45 on SDS-PAGE (M_r 50 kDa) and thirdly there are four amino acid substitutions in the first 25 N-terminal residues of PrtRII50. PrtRII50 has a Glu at position 8 instead of Gln, a Pro at position 17 instead of Ala, a Glu at position 22 instead of Gly and a Glu at position 25 instead of the Lys in PrtR45 (Fig. 8). These differences in size and the N-terminal amino acyl sequence were confirmed with the cloning and sequence analysis of the gene *prtRII* encoding the second Arg-specific proteinase.

The deduced amino acid sequence of the *prtRII* gene exhibits 98% identity with that of the recently reported *rgpB* gene from *P. gingivalis* ATCC 33277 (Nakayama, 1997) suggesting that both genes represent the same locus in two different strains. However, the sequence for the mature proteinase of the *rgpB* gene does not contain three of the N-terminal amino acyl substitutions found in the *prtRII* gene product and only has the Gln→Glu substitution at position 8. The substitutions at positions 17, 22 and 25 found in PrtRII50, that enabled the gene product to be unequivocally differentiated by N-terminal sequence analysis from the mature PrtR45 proteinase of the *prtR* (*rgpA*), were not found in the *rgpB*. In the current study the differences in N-terminal sequence and size of the mature proteinases enabled the differentiation of the discrete 50 kDa Arg-specific proteinase (PrtRII50) from the 45 kDa Arg-specific proteinase (PrtR45) found associated with adhesins. The assignment of the two proteinases (PrtR45 and PrtRII50) to the two genes (*prtR* and *prtRII* respectively) has enabled identification of a conserved motif in the two adhesin-associated proteinases (PrtR45 and PrtK48) not found in the discrete PrtRII50. As the conserved motif was also found in several adhesins of the *prtR* and *prtK* we propose that it is an adhesin binding motif involved in association of the *prtR* and *prtK* proteinases and adhesins into large complexes. This proposition is supported by the demonstration that a synthetic peptide corresponding to the conserved motif specifically binds to the TLCK-inactivated PrtR-PrtK complex.

The identification of the conserved motif PVXNLT.....LKWXAP in the adhesin binding motif 1 led us to propose that the complementary motif would be hydrophobic and negatively charged. Repeat motifs that were therefore hydrophobic and contatined negative residues were selected for synthesis. eg. TATTFEEDGVA (ABM 2, Table 1) and WKTIDADGDG (ABM 5,

Table 1). Other motifs selected for study were other repeated motifs of hydrophobic and/or charged and/or neutral polar residues eg. VYRDGTKIKE (ABM 2, Table 1), WEIRTVDLPAGTKYV (ABM 4, Table 1) and EFAPVQNLTGSA (ABM 6, Table 1).

On further examination of the alignment of the deduced amino acyl sequence of PrtRII50 with the catalytic domains of the PrtR45 Arg-specific proteinase and the PrtK48 Lys-specific proteinase some further interesting areas of similarity were revealed (Fig. 8). Although these three cysteine proteinases from *P. gingivalis* have no similarity with any of the other known families of cysteine proteinases it is possible to speculate on the identity of the catalytic residues since only one His residue and two Cys residues are conserved in the three sequence-related enzymes. The catalytic Cys, His dyad of these enzymes therefore is likely to consist of H^{440} of PrtRII50, the only conserved His in the three proteinases. The catalytic Cys is also likely to be one of the two conserved cysteinyl residues C^{473} and C^{484} in the three sequence-related proteinases.

EXAMPLE 2

Synthesis of Proteinase Active Site and Adhesin Binding Motif Peptides and Testing in a Murine Lesion Model.

The following peptides representative of the protease active sites and each adhesin binding motif listed in Table 1 were synthesised, conjugated and tested in the murine lesion model (Table 2).

Table 2. Origin and amino acid sequence of synthesised peptides

Origin	Amino acid sequence (single letter code)	Abbreviation
Proteinase Active Site Peptides		
PrtR45 (426-446)	FNGGISLANYTGHGSETAWGT	PAS1(R45)
PrtK48 (432-453)	LNTGVSFANYTAHGSETAWADP	PAS1(K48)
Adhesion Binding Motif Peptides		
PrtR45 (664-689)	PYQPVSNLTATTQGQKVTLKWDAPSTK	ABM1(R45)

PrtK39 (1580-1608)	SYTYTVYRDGTKIKEGLTATTFEEDGVAA	ABM2(K39)
PrtR44 (939-971)	VTLKWDAPNGTPNPNPNPNPNPNPGTTTLSESF	ABM3(R44)
PrtK44 (1296-1315)	WIERTVDLPAGTKYVAFRHY	ABM4(K44)
PrtR15 (1154-1169)	PAEWTTIDADGDGQGW	ABM5(R15)
PrtR44 (919-938)	EGSNEFAPVQNLTGSAVGQK	ABM6(R44)
Control Peptide		
PrtR27 (1432-1463)	ANEAKVVLAADNVWGDNTGYQFLLDADHNTFG	Control peptide

Materials

Unless otherwise stated chemicals were of peptide synthesis grade or its equivalent. O-Benzotriazole-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), 1hydroxybenzotriazole (HOBt), diisopropylethylamine (DIPEA), *N,N*-dimethylformamide (DMF), piperidine, trifluoroacetic acid (TFA) and 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acids were obtained from Auspep Pty Ltd (Melbourne, Australia). Triisopropylsilane (TIPS) and ethanedithiol (EDT) were obtained from Aldrich (New South Wales, Australia). 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was obtained from Sigma Chemical Company (New South Wales, Australia). Phenol and diethyl ether were obtained from BDH (Poole, UK).

Solid-Phase Peptide Synthesis

Peptides were synthesised manually or using a 431A ABI peptide synthesiser. Standard solid-phase peptide synthesis protocols for Fmoc chemistry were used throughout. Peptides were assembled as the carboxyamide form using Fmoc-Pal-Peg-PS resin (PerSeptive Biosystems Inc., Framingham, MA). Coupling was accomplished with HBTU/HOBt activation using 4 equiv of Fmoc-amino acid and 6 equiv of DIPEA. The Fmoc group was removed by 2% v/v DBU in DMF containing 2% v/v piperidine. Cleavage of peptides from the resin support was performed using TFA:phenol:TIPS:EDT:water (92:2:2:2:2) cleavage cocktail for 2.5 hours or 4 hours depending on the arginine content of the peptide. After cleavage the resin was removed by filtration and the filtrate concentrated to approximately 1mL under a stream of nitrogen. After the peptide products were precipitated in cold ether, they were centrifuged and washed three

times. The peptide precipitate was then dissolved in 5 to 10 mL of water containing 0.1% v/v TFA and insoluble residue removed by centrifugation.

Synthesis of S-Acetylmercaptoacetic acid Peptides

Resins bearing peptides were swollen in DMF and the N-terminal Fmoc group removed by 2% v/v DBU in DMF containing 2% v/v piperidine. S-Acetylmercaptoacetic acid (SAMA) group was introduced onto the N-terminal amino group using 5 equiv of SAMA-OPfp and 5 equiv of HOBt. The reaction was monitored by the trinitrobenzene sulphonic acid (TNBSA) test. When a negative TNBSA test was returned the resin was washed (5 x DMF, 3 x DCM and 3 x diethyl ether). The resin was dried under vacuum and the SAMA-peptides cleaved from the resin support as described above.

Peptide Purification

Purification of synthesized peptides was performed using a Brownlee C18 Aquapore ODS column (250 x 100 mm) installed in a Waters HPLC system. Chromatograms were developed at a flow rate of 5 mL/min using 0.1% v/v TFA in water (solvent A) and 0.1% v/v TFA in 90% aqueous acetonitrile (solvent B) as the limit buffer. Peptides were eluted with a gradient of 10-30% solvent B formed over 40 min. Analytical HPLC was carried out using a Brownlee C8 Aquapore RP-300 column (220 x 4.6 mm) installed in a Applied Biosytems HPLC system. Chromatograms were developed using solvent A and solvent B at a flow rate of 1 mL/min and a 0-100% linear gradient of solvent B formed over 30 min. Material eluted from the columns was detected by determining the absorbance at 214 nm. Peptide fractions were pooled and lyophilised. Peptides were analysed by mass spectrometry using a PerSeptive Biosystems Voyager DE MALDI-TOF.

Conjugation of SAMA-Peptides to Diphtheria Toxoid

Diphtheria toxoid (DT) was obtained from Dr I. Barr (CSL Pty. Ltd. Melbourne, Australia) which contained 9 equivalent amino groups per 62 kDa molecule. To a solution containing 10mg/mL of DT in phosphate-buffered saline (0.1M sodium phosphate, 0.9% NaCl; pH 7.4) was added 0.1mL of a 1% w/v solution m-maleimido benzoyl-N-hydroxysuccinimide ester (MBS) in DMF. After 30 mins unreacted MBS was removed and MBS modified DT collected by gel filtration

using a PD10 column (Pharmacia, NSW, Australia) equilibrated in conjugation buffer (0.1M sodium phosphate, 5mM EDTA; pH 6.0). Purified SAMA-peptide (1.3μmole) was dissolved in 200μL 6M guanidine HCl containing 0.5M Tris; 2mM EDTA, pH6 and diluted with 800μL MilliQ water and deprotected in-situ by addition of 25μL of 2M NH_2OH (40 equiv) dissolved in MilliQ water. The collected MBS-DT was immediately reacted with deprotected SAMA-peptide and stirred for one hour at room temperature. The peptide-DT conjugate was separated from unreacted peptide by gel filtration using a PD10 column equilibrated in PBS pH 7.4 and lyophilised. The reaction was monitored using the Ellmans test. The conjugation yields of SAMA-peptides to MBS-DT ranged from 34% to 45% indicating that 3 to 4 peptides were coupled per DT molecule.

Immunization and Murine Lesion Model Protocols

BALB/c mice 6-8 weeks old were immunised subcutaneously with either 50μg of the peptide-DT conjugate, 50μg of DT or 2×10^9 formalin killed cells of *Porphyromonas gingivalis* strain 33277 emulsified in complete Freund's adjuvant (CFA). After 30 days the mice were injected subcutaneously with antigen (either 50μg of the peptide-DT conjugate, 50μg of DT or 2×10^9 formalin killed cells of *Porphyromonas gingivalis* strain 33277) emulsified in incomplete Freund's adjuvant (IFA) and then bled from the retrobulbar plexus 12 days later. All mice were challenged with 8×10^9 cells of P. gingivalis (200μL) by subcutaneous injection in the abdomen and weighed and lesion size measured over 10 days. Lesion sizes are expressed as mm^2 and were statistically analysed using a Kruskal-Wallis one-way ANOVA and Mann-Whitney U-Wilcoxon rank sum W test.

The peptide-DT conjugates were used to immunise BALB/c mice to evaluate their efficacy in protecting against *Porphyromonas gingivalis* challenge in the murine lesion model. Figure 6 shows that mice that were immunised with the carrier protein diphtheria toxoid alone had similar average lesion sizes to the mice immunised with adjuvant alone (controls). This indicates that DT alone does not provide protection against *P. gingivalis* and moreover that any protection provided by peptide-DT conjugates was attributable to the immune response induced by the peptide. The control peptide-DT conjugate did not provide protection against *P. gingivalis* as the average lesion size was not significantly different to that of mice immunised

with DT or adjuvant alone (controls). Immunisation with both the proteinase active site peptides conjugated to DT (PAS1(R45) and PAS1(K48)) Significantly reduced lesion size resulting from *P. gingivalis* challenge relative to the DT controls(Table 3). All the adhesin binding motif peptides when used as immunogens reduced lesion size however, only ABM1(R45), ABM2(K39) and ABM3(R44) attained significance ($p<0.05$) with the number of animals used (Table 3)

The results demonstrate the efficacy of the PrtR-PrtK proteinase active site peptides and adhesin binding motif peptides when used as immunogens in preventing challenge with *P. gingivalis* in the murine lesion model. These results therefore suggest that these peptides may have utility as vaccines in the prevention of *P. gingivalis*-associated disease (e.g Periodontitis) in humans.

Antisera against the PAS1 peptides inhibited both Arg- and Lys-specific proteolytic activity which therefore may explain the excellent protection conferred by immunisation with these peptides. The inhibition of proteolytic activity by the anti-PAS1 antisera suggests that these antibodies may have utility in a mouthwash, toothpaste or other intra-oral delivery vehicle to neutralise the *P. gingivalis* proteases and their damaging effects. Similarly, antisera against the adhesin binding motifs, particularly ABM1, ABM2 and ABM3 may have utility in oral care products and pharmaceuticals to block adherence and therefore colonisation of *P. gingivalis*.

Table 3. Maximum Lesion size and significance of peptide-diphtheria conjugates.

	DT[d]	ABM1(R45)-DT	ABM2(K39)-DT	ABM3(R44)-DT	ABM4(K44)-DT	ABM5(R15)-DT	ABM6(R44)-DT	PAS1(R45)-DT	PAS1(K48)-DT	Control peptide -DT	FK 33277[e]
Maximum lesion size (mm^2)	33.59 ±18.77[a]	10.42 ±11.7	12.63 ±10.89	12.27 ±4.68	18.83 ±18.87	14.79 ±10.04	15.22 ±11.55	10.46 ±4.08	9.28 ±10.36	36.61 ±34.92	13.78 ±12.55
Significance[b]	-	$p < 0.05$	$p < 0.05$	$P < 0.05$	N/S[c]	N/S[c]	N/S[c]	$P < 0.05$	$p < 0.05$	N/S[c]	$p < 0.05$

a = standard deviation n=5,6
b = Mann-Whitney U test.
c = no significant difference
d = Diphtheria Toxoid
e = formalin killed *Porphyromonas gingivalis* strain 33277

EXAMPLE 3

(1) Synthesis of Peptide Antigens and Multiple Constructs

The peptides of Table 1 were synthesized using standard Fmoc or tBoc synthesis strategies and multipeptide constructs were synthesized using the strategies outlined in Figs. 1-5.

(2) Preparation of Antibodies

Serum antibodies were obtained by immunising horses, rabbits, sheep or dairy cows.

Immunizations were carried out using standard procedures. The initial immunisation was with a mixture of the antigen and Freund's incomplete adjuvant. The antibodies could be recovered from the animals serum or milk using standard procedures.

EXAMPLE 4

Methods for using antigenic peptides in diagnostic immunoassays.

The *P. gingivalis* peptide antigens described herein can be synthesized for use as immunogens in vaccine formulations; and as antigens for diagnostic assays or for generating *P. gingivalis*-specific antisera of therapeutic and/or diagnostic value.

The peptides disclosed in Table 1 can be synthesized individually or chemically-linked using any one of a number of strategies well known in the art. Examples of some strategies which can be used are set out in Figs. 1 - 5. The peptides can be synthesized using one of the several methods of peptide synthesis known in the art including standard solid phase peptide synthesis using tertbutyloxycarbonyl amino acids (Mitchell *et al.*, 1978, *J. Org. Chem.* 43:2845-2852), using 9-fluorenylmethyloxycarbonyl amino acids on a polyamide support (Dryland *et al.*, 1986, J. *Chem. So. Perkin Trans.* I, 125-137); by pepscan synthesis (Geysen *et al.*, 1987, *J. Immunol. Methods* 03:259; 1984, *Proc. Natl. Acad. Sci. USA* 81:3998); or by standard liquid phase peptide synthesis. Modification of the peptides or oligopeptides, such as by deletion and substitution of amino acids (and including extensions and additions to amino acids) and in other ways, may be made so as to not substantially detract from the immunological properties of the peptide or oligopeptide. In

particular, the amino acid sequences of the antigens described herein, may be altered by replacing one or more amino acids with functionally equivalent amino acids resulting in an alteration which is silent in terms of an observed difference in the physicochemical behaviour of the peptide, or oligopeptide or chimera. Functionally equivalent amino acids are known in the art as amino acids which are related and/or have similar polarity or charge. Thus, an amino acid sequence which is substantially that of the amino acid sequences depicted in the Sequence Listing herein, refers to an amino acid sequence that contains substitutions with functionally equivalent amino acids without changing the primary biological function of the peptide, oligopeptide or chimera.

Purified synthetic peptides may be used as antigens in immunoassays for the detection of *P. gingivalis*-specific antisera present in the body fluid of an individual suspected of having an infection caused by *P. gingivalis*. The detection of antigens or related peptides in immunoassays, includes any immunoassay known in the art including, but not limited to, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), "sandwich" assay, precipitin reaction, agglutination assay, fluorescent immunoassay, and chemiluminescence-based immunoassay.

EXAMPLE 5

Methods and compounds for vaccine formulations related to synthetic peptide antigens and multipeptide constructs.

This embodiment of the present invention is to provide peptide antigens of Table 1 to be used as immunogens in a prophylactic and/or therapeutic vaccine for active immunization to protect against or treat infections caused by *P. gingivalis*. For vaccine purposes, an antigen of *P. gingivalis* comprising a synthetic peptide construct should be immunogenic, and induce functional antibodies directed to one or more surface-exposed epitopes on intact bacteria, wherein the epitope(s) are conserved amongst strains of *P. gingivalis*.

In one illustration of the invention, the dipeptide PAS1-PAS2 construct (Fig. 4) having the properties desirable of a vaccine antigen, the dipeptide construct can be synthesized using the method described herein in Example 3.

The synthetic peptide is included as the relevant immunogenic material in the vaccine formulation, and in therapeutically effective amounts, to induce an immune response. Many methods are known for the introduction of a vaccine formulation into the human or animal to be vaccinated. These include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, ocular, intranasal, and oral administration. The vaccine may further comprise a physiological carrier such as a solution, a polymer or liposomes; and an adjuvant, or a combination thereof.

Various adjuvants are used in conjunction with vaccine formulations. The adjuvants aid by modulating the immune response and in attaining a more durable and higher level of immunity using smaller amounts of vaccine antigen or fewer doses than if the vaccine antigen were administered alone. Examples of adjuvants include incomplete Freund's adjuvant (ISA), Adjuvant 65 (containing peanut oil, mannide monooleate and aluminum monostrearate), oil emulsions, Ribi adjuvant, the pluronic polyols, polyamines, Avridine, Quil A, saponin, MPL, QS-21, and mineral gels such as aluminum hydroxide, aluminum phosphate, etc.

Another embodiment of this mode of the invention involves the production of antigen-specific amino acid sequences as a hapten, i.e. a molecule which cannot by itself elicit an immune response. In such case, the hapten may be covalently bound to a carrier or other immunogenic molecule which will confer immunogenicity to the coupled hapten when exposed to the immune system. Thus, such a antigen-specific hapten linked to a carrier molecule may be the immunogen in a vaccine formulation.

As an alternative to active immunization, immunization may be passive, i.e. immunization comprising administration of purified immunoglobulin containing antibody against synthetic peptides.

EXAMPLE 6

The following is an example of a proposed toothpaste formulation containing anti-peptide antibodies.

Ingredient	% w/w
Dicalcium phosphate dihydrate	50.0
Glycerol	20.0
Sodium carboxymethyl cellulose	1.0
Sodium lauryl sulphate	1.5
Sodium lauroyl sarconisate	0.5
Flavour	1.0
Sodium saccharin	0.1
Chlorhexidine gluconate	0.01
Dextranase	0.01
Goat serum containing anti-peptide Abs	0.2
Water	balance

EXAMPLE 7

The following is an example of a proposed toothpaste formulation.

Ingredient	% w/w
Dicalcium phosphate dihydrate	50.0
Sorbitol	10.0
Glycerol	10.0
Sodium carboxymethyl cellulose	1.0
Sodium lauryl sulphate	1.5
Sodium lauroyl sarconisate	0.5
Flavour	1.0
Sodium saccharin	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	0.01
Dextranase	0.01
Bovine serum containing anti-peptide Abs	0.2
Water	balance

EXAMPLE 8

The following is an example of a proposed toothpaste formulation.

Ingredient	% w/w
Dicalcium phosphate dihydrate	50.0
Sorbitol	10.0
Glycerol	10.0
Sodium carboxymethyl cellulose	1.0
Lauroyl diethanolamide	1.0
Sucrose monolaurate	2.0
Flavour	1.0
Sodium saccharin	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	0.01
Dextranase	0.01
Bovine milk Ig containing anti-peptide Abs	0.1
Water	balance

EXAMPLE 9

The following is an example of a proposed toothpaste formulation.

Ingredient	% w/w
Sorbitol	22.0
Irish moss	1.0
Sodium Hydroxide (50%)	1.0
Gantrez	19.0
Water (deionised)	2.69
Sodium Monofluorophosphate	0.76
Sodium saccharine	0.3
Pyrophosphate	2.0
Hydrated alumina	48.0
Flavour oil	0.95
anti-peptide mouse monoclonal	0.3
sodium lauryl sulphate	2.00

EXAMPLE 10

The following is an example of a proposed liquid toothpaste formulation.

Ingredient	% w/w
Sodium polyacrylate	50.0
Sorbitol	10.0
Glycerol	20.0
Flavour	1.0
Sodium saccharin	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	0.01
Ethanol	3.0
Equine Ig containing anti-peptide Ab	0.2
Linolic acid	0.05
Water	balance

EXAMPLE 11

The following is an example of a proposed mouthwash formulation.

Ingredient	% w/w
Ethanol	20.0
Flavour	1.0
Sodium saccharin	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	0.01
Lauroyl diethanolamide	0.3
Rabbit Ig containing anti-peptide-Ab	0.2
Water	balance

EXAMPLE 12

The following is an example of a proposed mouthwash formulation.

Ingredient	% w/w
Gantrez S-97	2.5
Glycerine	10.0
Flavour oil	0.4
Sodium monofluorophosphate	0.05
Chlorhexidine gluconate	0.01
Lauroyl diethanolamide	0.2
Mouse anti-peptide monoclonal	0.3
Water	balance

EXAMPLE 13

The following is an example of a proposed lozenge formulation.

Ingredient	% w/w
Sugar	75-80
Corn syrup	1-20
Flavour oil	1-2
NaF	0.01-0.05
Mouse anti-peptide monoclonal	0.3
Mg stearate	1-5
Water	balance

EXAMPLE 14

The following is an example of a proposed gingival massage cream formulation.

Ingredient	% w/w
White petrolatum	8.0
Propylene glycol	4.0
Stearyl alcohol	8.0
Polyethylene Glycol 4000	25.0
Polyethylene Glycol 400	37.0
Sucrose monostearate	0.5
Chlorohexidine gluconate	0.1
Mouse anti-peptide monoclonal	0.3
Water	balance

EXAMPLE 15

The following is an example of a proposed chewing gum formulation.

Ingredient	% w/w
Gum base	30.0
Calcium carbonate	2.0
Crystalline sorbitol	53.0
Glycerine	0.5
Flavour oil	0.1
Mouse anti-peptide monoclonals	0.3
Water	balance

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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CLAIMS:

1. A composition for use in raising an immune response against *Porphyromonas gingivalis*, the composition including a suitable adjuvant and/or acceptable carrier or excipient and at least one peptide selected from the group consisting of:-

FNGGISLANYTGHGSETAWGT;
LNTGVSFANYTAHGSETAWADP;
FDVACVNGDFLFSMPCFAEALMRA;
IGNCCITAQFDYVQPCFGEVITRV;
GEPNPYQPVSNLTATTQGQKVTLKWDAPSTK;
EGSNEFAPVQNLTGSAVGQKVTLKWDAPNGT;
VNSTQFNPVKNLKAQPDGGDVVLKWEAPSAK;
GEPSPYQPVSNLTATTQGQKVTLKWEAPSAK;
EGSNEFAPVQNLTGSSVGQKVTLKWDAPNGT;
VNSTQFNPVQNLTAEQAPNSMDAILKWNAPASK;
QFNPVQNLTGSAVGQKVTLKWDAPNGT;
FAHVQNLTGSAVGQKVTLKWDAPNGT;
FAPVQNLQWSVSGQTVTLTWQAPASD;
QFNPVQNLTAEQAPNSMDAILKWNAPASK;
DYTYTVYRDGTKIKEGLTATTFEEDGVAT;
DYTYTVYRDGTKIKEGLTETTFEEDGVAT;
SYTYTVYRDGTKIKEGLTETTYRDAGMSA;
SYTYTVYRDGTKIKEGLTATTFEEDGVAA;
DYTYTVYRDGTKIKEGLTETTFEEDGVAT;
SYTYTIYRNNTQIASGVTETTYRDPDLAT;
DYTYTVYRDNVVIAQNLAATTFNQENVAP;
SYTYTIYRNNTQIASGVTETTYRDPDLAT;
PNGTPNPNPNPNPNPNPGTTTLSESF;
PNGTPNPNPNPNPNPGTTLSESF;
PNGTPNPNPNPNPGTTTLSESF;
PNGTPNPNPGTTTLSESF;
WIERTVDLPAGTKYVAFRHY;
WRQKTVDLPAGTKYVAFRHF;
WYQKTVQLPAGTKYVAFRHF;

ERTIDLSAYAGQQVYLAFRHF;
PAEWTTIDADGDGQGW;
PASWKTIDADGDGHGW;
PASWKTIDADGDGNNW;
PSSWKTIDADGDGNNW;
PNGWTMIDADGDGHNW;
EGSNEFAPVQNLTGSAVGQK;
GEPNPYQPVSNLTATTQGQK;
EGSNEFAPVQNLTGSSVGQK;
GEPSPYQPVSNLTATTQGQK;
NSTQFNPVQNLTAEQAPNS;
EGSNEFAHVQNLTGSAVGQK;
DPVQFNPVQNLTGSAVGQK;
EGGNEFAPVQNLQWSVSGQT;
NPTQFNPVQNLTAEQAPNS;
GNHEYCVEVKYTAGVSPKVCKDVTV;
GNHEYCVEVKYTAGVSPKKCVNVTV;
SHEYCVEVKYTAGVSPKVCVD;
GNHEYCVEVKYTAGVSPKKCVNVTV;
GNHEYCVEVKYTAGVSPKVCVNVTI;
GQYNYCVEVKYTAGVSPKVCKDVTV; and
GNHEYCVEVKYTAGVSPEVCVNVTV.

2. A composition as claimed in claim 1 in which the composition includes at least one peptide selected from the group consisting of:-

FNGGISLANYTGHGSETAWGT;
LNTGVSFANYTAHGSETAWADP;
PYQPVSNLTATTQGQKVTLKWDAPSTK;
SYTYTVYRDGTKIKEGLTATTFEEDGVAA;
VTLKWDAPNGTPNPNPNPNPNPNPGTTTLSESF;
WIERTVDLPAGTKYVAFRHY;
PAEWTTIDADGDGQGW; and
EGSNEFAPVQNLTGSAVGQK.

3. A composition as claimed in claim 1 or claim 2 in which the composition includes more than one peptide.

4. A composition as claimed in claim 3 in which the composition includes one or more multimers of different peptides.

5. A peptide selected from the group consisting of:-

FNGGISLANYTGHGSETAWGT;
LNTGVSFANYTAHGSETAWADP;
FDVACVNGDFLFSMPCFAEALMRA;
IGNCCITAQFDYVQPCFGEVITRV;
GEPNPYQPVSNLTATTQGQKVTLKWDAPSTK;
EGSNEFAPVQNLTGSAVGQKVTLKWDAPNGT;
VNSTQFNPVKNLKAQPDGGDVVLKWEAPSAK;
GEPSPYQPVSNLTATTQGQKVTLKWEAPSAK;
EGSNEFAPVQNLTGSSVGQKVTLKWDAPNGT;
VNSTQFNPVQNLTAEQAPNSMDAILKWNAPASK;
QFNPVQNLTGSAVGQKVTLKWDAPNGT;
FAHVQNLTGSAVGQKVTLKWDAPNGT;
FAPVQNLQWSVSGQTVTLTWQAPASD;
QFNPVQNLTAEQAPNSMDAILKWNAPASK;
DYTYTVYRDGTKIKEGLTATTFEEDGVAT;
DYTYTVYRDGTKIKEGLTETTFEEDGVAT;
SYTYTVYRDGTKIKEGLTETTYRDAGMSA;
SYTYTVYRDGTKIKEGLTATTFEEDGVAA;
DYTYTVYRDGTKIKEGLTETTFEEDGVAT;
SYTYTIYRNNTQIASGVTETTYRDPDLAT;
DYTYTVYRDNVVIAQNLAATTFNQENVAP;
SYTYTIYRNNTQIASGVTETTYRDPDLAT;
PNGTPNPNPNPNPNPGTTTLSESF;
PNGTPNPNPNPNPGTTLSESF;
PNGTPNPNPNPNPGTTTLSESF;
PNGTPNPNPGTTTLSESF;
WIERTVDLPAGTKYVAFRHY;
WRQKTVDLPAGTKYVAFRHF;

WYQKTVQLPAGTKYVAFRHF;
ERTIDLSAYAGQQVYLAFRHF;
PAEWTTIDADGDGQGW;
PASWKTIDADGDGHGW;
PASWKTIDADGDGNNW;
PSSWKTIDADGDGNNW;
PNGWTMIDADGDGHNW;
EGSNEFAPVQNLTGSAVGQK;
GEPNPYQPVSNLTATTQGQK;
EGSNEFAPVQNLTGSSVGQK;
GEPSPYQPVSNLTATTQGQK;
NSTQFNPVQNLTAEQAPNS;
EGSNEFAHVQNLTGSAVGQK;
DPVQFNPVQNLTGSAVGQK;
EGGNEFAPVQNLQWSVSGQT;
NPTQFNPVQNLTAEQAPNS;
GNHEYCVEVKYTAGVSPKVCKDVTV;
GNHEYCVEVKYTAGVSPKKCVNVTV;
SHEYCVEVKYTAGVSPKVCVD;
GNHEYCVEVKYTAGVSPKKCVNVTV;
GNHEYCVEVKYTAGVSPKVCVNVTI;
GQYNYCVEVKYTAGVSPKVCKDVTV; and
GNHEYCVEVKYTAGVSPEVCVNVTV.

6. A peptide as claimed in claim 5 selected from the group consisting of:-

FNGGISLANYTGHGSETAWGT;
LNTGVSFANYTAHGSETAWADP;
PYQPVSNLTATTQGQKVTLKWDAPSTK;
SYTYTVYRDGTKIKEGLTATTFEEDGVAA;
VTLKWDAPNGTPNPNPNPNPNPNPGTTTLSESF;
WIERTVDLPAGTKYVAFRHY;
PAEWTTIDADGDGQGW; and
EGSNEFAPVQNLTGSAVGQK.

7. An antibody preparation comprising antibodies specifically directed against a peptide as claimed in claim 5 or claim 6.

8. An antibody preparation as claimed in claim 7 in which the antibodies are polyclonal antibodies.

9. An antibody preparation as claimed in claim 7 in which the antibodies are monoclonal antibodies.

10. A method of treating a subject suffering from *Porphyromonas gingivalis* infection, the method comprising administering to the subject an effective amount of a composition as claimed in any one of claims 1 to 4 or a peptide as claimed in claim 5 or claim 6.

11. A method according to claim 10 in which the composition or peptide is administered as a mouth wash or as a dentifrice.

12. A method of treating a subject suffering from *Porphyromonas gingivalis* infection, the method comprising administering to the subject an effective amount of an antibody preparation as claimed in any one of claims 7 to 9.

13. A method as claimed in claim 12 in which the antibody preparation is administered as a mouth wash or as a dentifrice.

14. A method of reducing the prospect of *P. gingivalis* infection in an individual and/or severity of disease, the method comprising administering to the individual an amount of a composition as claimed in any one of claims 1 to 4 effective to induce an immune response in the individual directed against *P. gingivalis*.

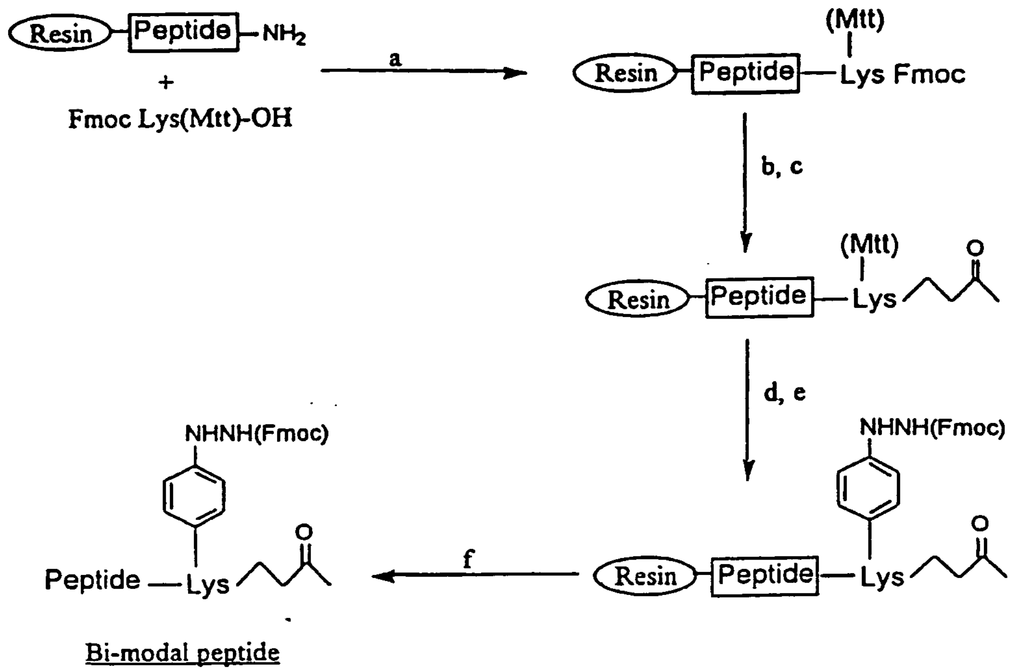

Resin
Peptide
NH2
+
Fmoc Lys(Mtt)-OH
a
(Mtt)
Lys Fmoc
b, c
Lys
d, e
NHNH(Fmoc)
f
Peptide — Lys
Bi-modal peptide

Figure 1

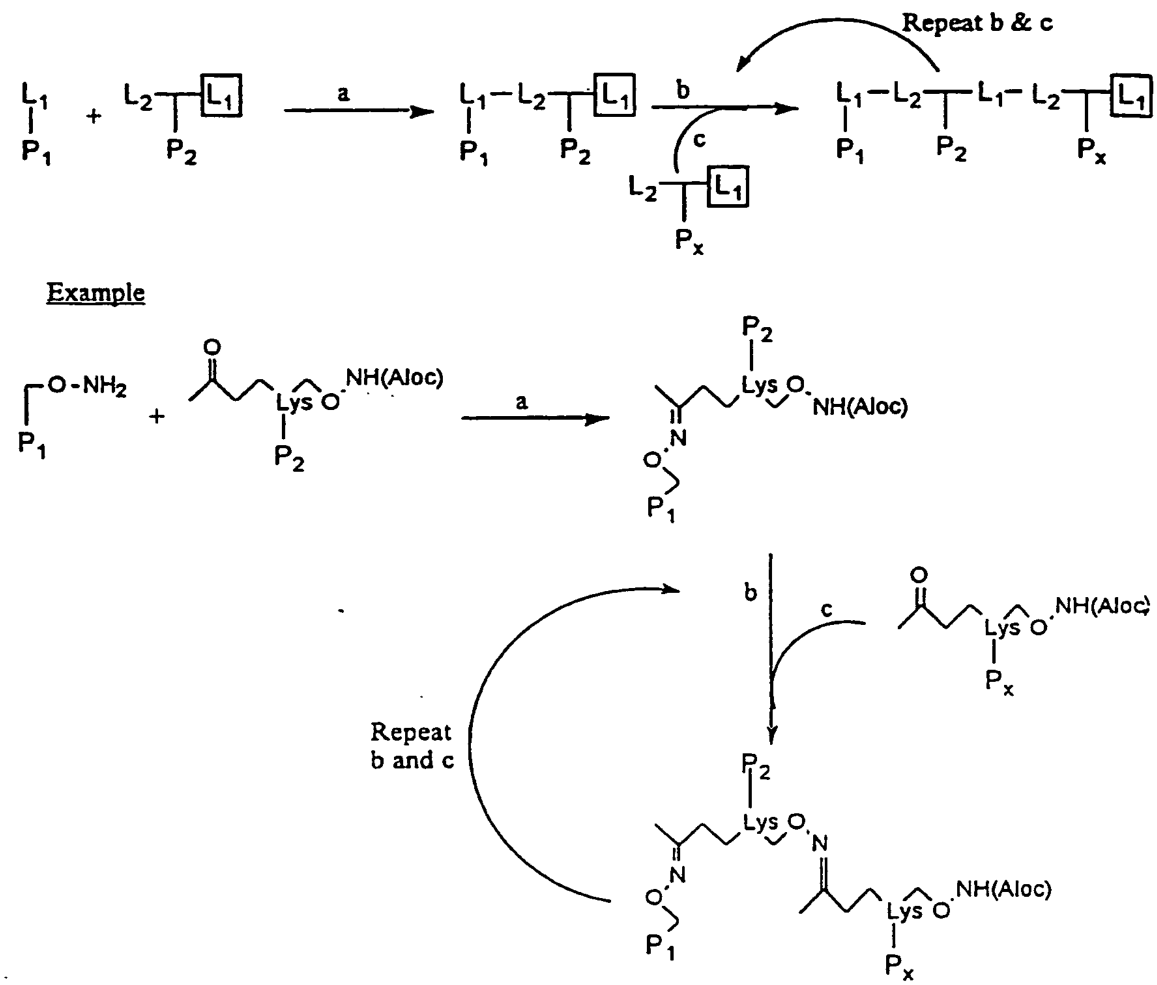
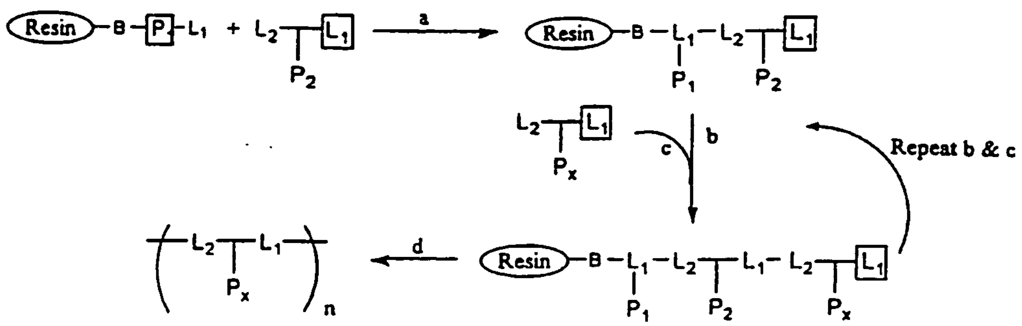


Figure 2

PCT/AU98/00311



Example

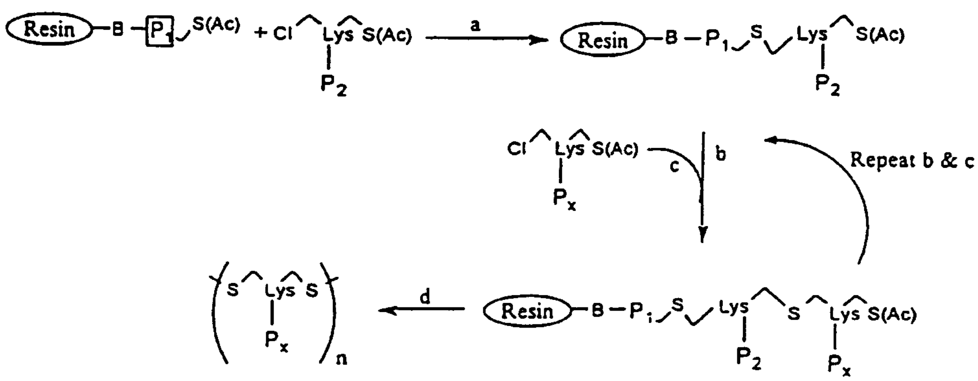
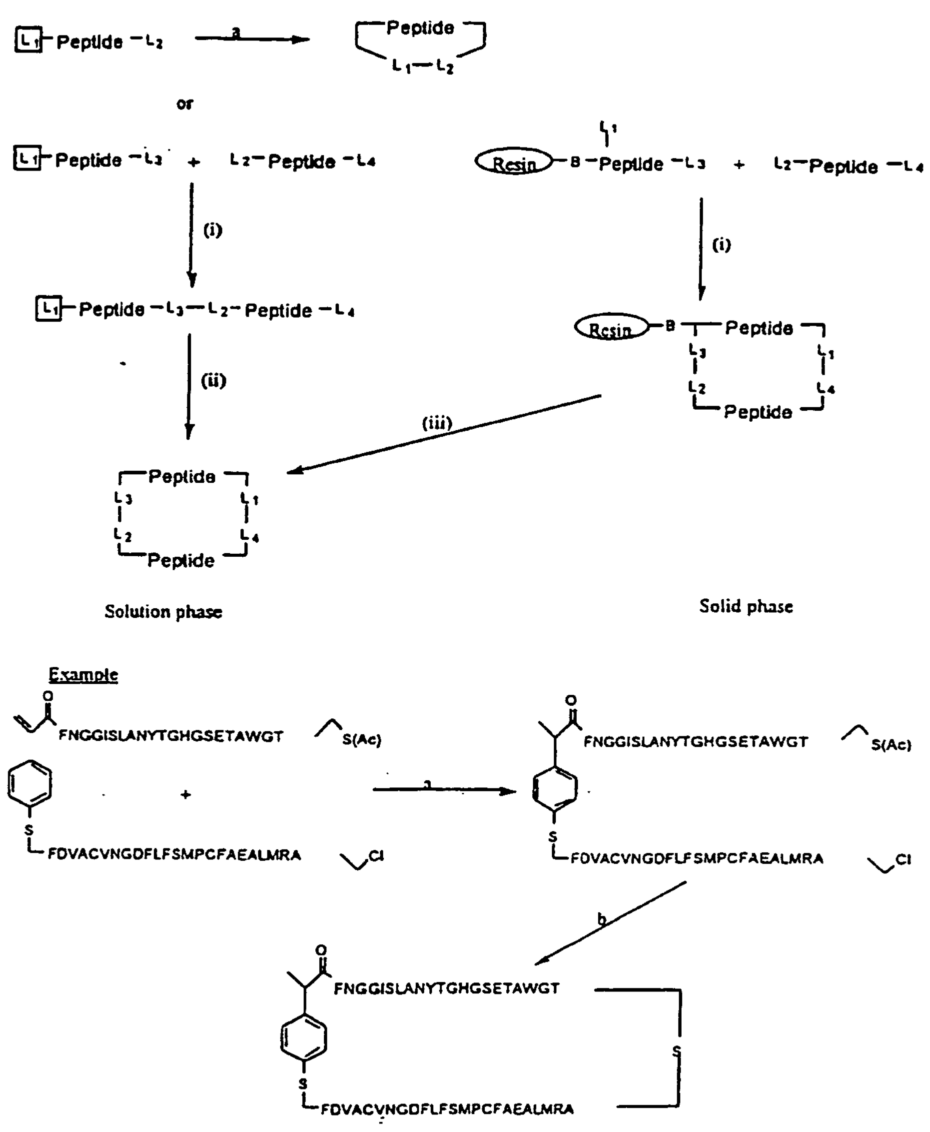


Figure 3



Peptide
L1
L2
a
or
L3
L4
(i)
(ii)
(iii)
Resin
B
Solution phase
Solid phase
Example
FNGGISLANYTGHGSETAWGT
S(Ac)
FDVACVNGDFLFSMPCFAEALMRA
Cl
S
b

Figure 4

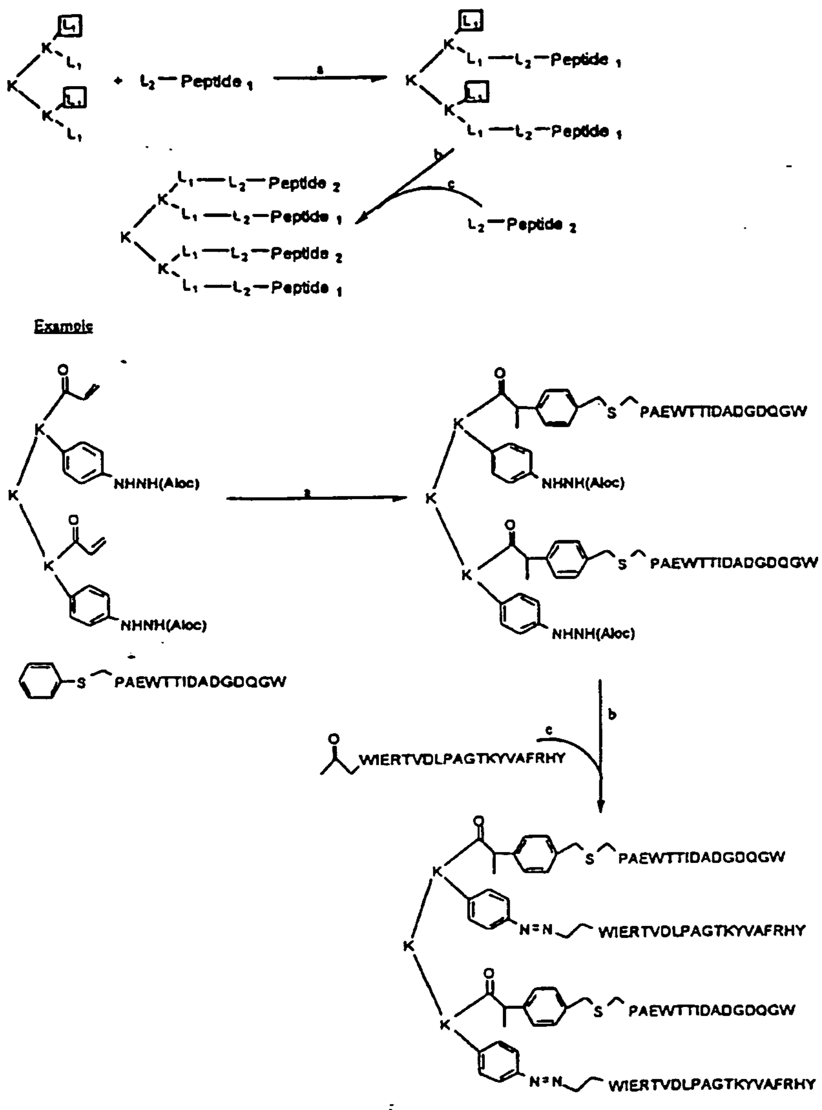
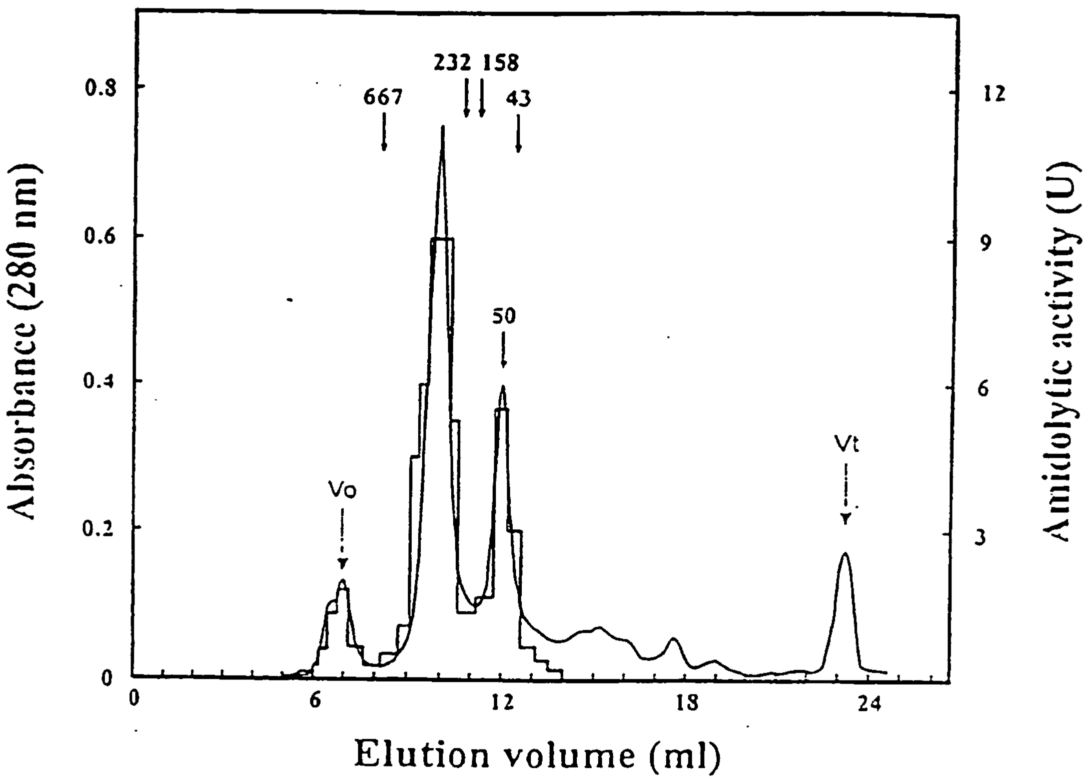

L2—Peptide 1
a
b
c
L1—L2—Peptide 1
L1—L2—Peptide 2
L2—Peptide 2
Example
NHNH(Aloc)
PAEWTTIDADGDQGW
WIERTVDLPAGTKYVAFRHY
N=N

Figure 5



0.8
0.6
0.4
0.2
0
Absorbance (280 nm)
667
232 158
43
50
Vo
Vt
12
9
6
3
Amidolytic activity (U)
0
6
12
18
24
Elution volume (ml)

Figure 6

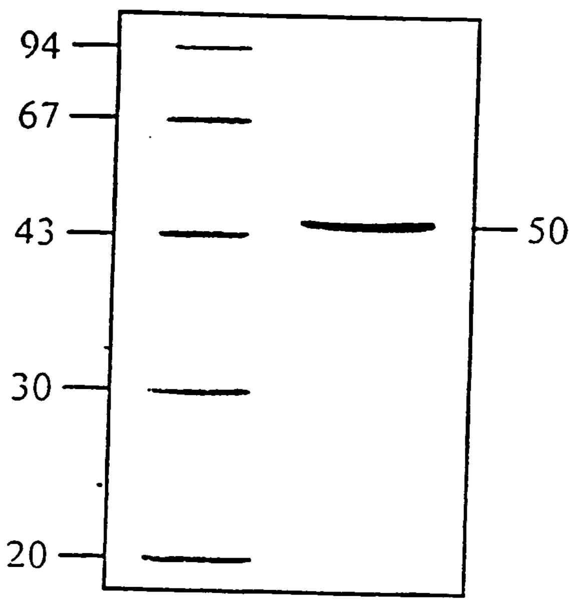


Figure 7

```
            230                                               275
PrtRII50    ..YTPVEEKENG..RMIVIVPKKYEEDIEDFVDWKNQRGLRTEVKVAEDI
PrtR45      ..*******Q**..******A****G**K*********************
PrtK48      DV**DHGDLY*TPV**L*VAGA*FK*ALKPWLT**A*K*FYLD*HYTDEA

            276                                               321
PrtRII50    ASPVTANAIQQFVKQEYEKE...GNDLTYVLLVGDHKDIPA.KITPGIKS
PrtR45      ********************...**********I*******.********
PrtK48      EVGT*NASIKA*IHKK*NDGLAASAAPVFLA****TDV*SGE*GKKTK*V

            322                                               371
PrtRII50    DQVYGQIVGNDHYNEVFIGRFSCESKEDLKTQIDRTIHYERNITTEDKWL
PrtR45      **************************************************
PrtK48      TDL*YSA*DG*YFP*MYTF*M*AS*P*E*TNI**KVLM**KATMPDKSY*

            372                                               421
PrtRII50    GQALCIASAEGGPSADNGESDIQHENIIANLLTQYGYTKIIKCYDPGVTP
PrtR45      **************************V***********************
PrtK48      EKV*L**G*DYSWNSQV*QPT*KYG.MQYYYNQEH***DVYNYLKAPY*.

            422                                               471
PrtRII50    KNIIDAFNGGISLANYTGHGSETAWGTSHFGTTHVKQLTNSNQLPFIFDV
PrtR45      **************************************************
PrtK48      .GCYSHL*T*V*F****A*******ADPLLT*SQL*A***KDKYFLAIGN

            472                                               521
PrtRII50    ACVNGDFLYNVPCFAEALMRAQKDGKPTGTVAIIASTINQSWASPMRGQD
PrtR45      ********FSM***************************************
PrtK48      C*ITAQ*D*VQ***G*VIT*....V*EK*AY*Y*G*SP*SY*GEDYYWSV

            522                                               561
PrtRII50    EMNEILCEKHP..NNIKRTFGGVTMNGMFAMVEKYKKDGEKM........
PrtR45      ***********..*****************************........
PrtK48      GA*AVFGVQPTFEGTSMGSYDATFLEDSYNTVNSIMWA*NLAATHAGNIG

            562                                               601
PrtRII50    ..........LDTWTVFGDPSLLVRTLVPTKMQVTAPANISASAQTFEVA
PrtR45      ..........****************************Q*NLTDASVN*S
PrtK48      NITHIGAHYYWEAYH*L**G*VMPYRAM*KTNTY*L**SLPQNQASYSIQ

            602                                               650
PrtRII50    CDYNGAIATLSDDGDMVGTAIVK.DGKAIIKLNESIADETNLTLTVVGYN
PrtR45      *********I*AN*K*F*S*V*E.N*T*T*N*.TGLTN*ST*********
PrtK48      ASAGSYV*.I*K**VLY**GVANAS*V*TVSMTKQ*TENG*YDVVITRS*

            651                                               699
PrtRII50    KVTVIKDVKVEGTSIA.DVANDKPYTVAVSGKTITVESPAAGLTIFDMNG
PrtR45      *E****TINTN*EPNPYQPVSNLTA*TQGQKV*LKWDA*STKTNATTNTA
PrtK48      YLP***QIQ*.*EPSPYQPVSNLTA*TQGQKV*LKW*A*S*KKAEGSREV

            700                                  736
PrtRII50    RRVATAKNRMVFEAQNGVYAVRIATEGKTYTEKVIVK
PrtR45      *SVDGIRELVLLSVSDAPELL*...............
PrtK48      K*IGDG....L*VTIEPAND**...............
```

Figure 8

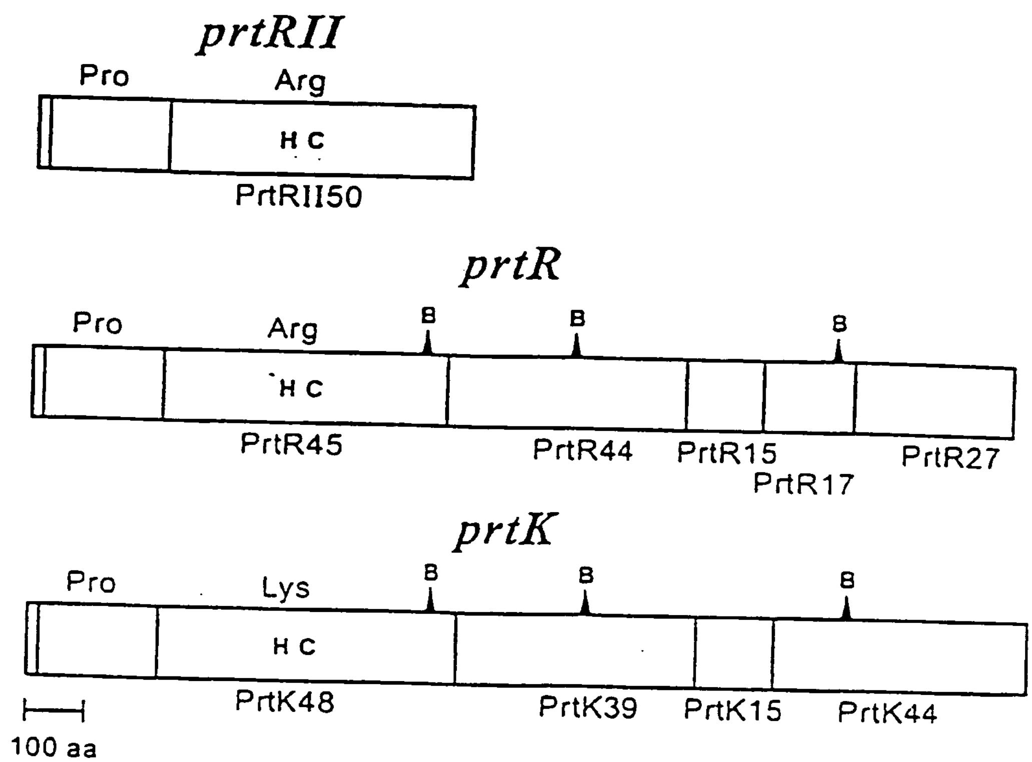

prtRII
Pro
Arg
H C
PrtRII50
prtR
Pro
Arg
B
B
B
H C
PrtR45
PrtR44
PrtR15
PrtR17
PrtR27
prtK
Pro
Lys
B
B
B
H C
PrtK48
PrtK39
PrtK15
PrtK44
100 aa

Figure 9

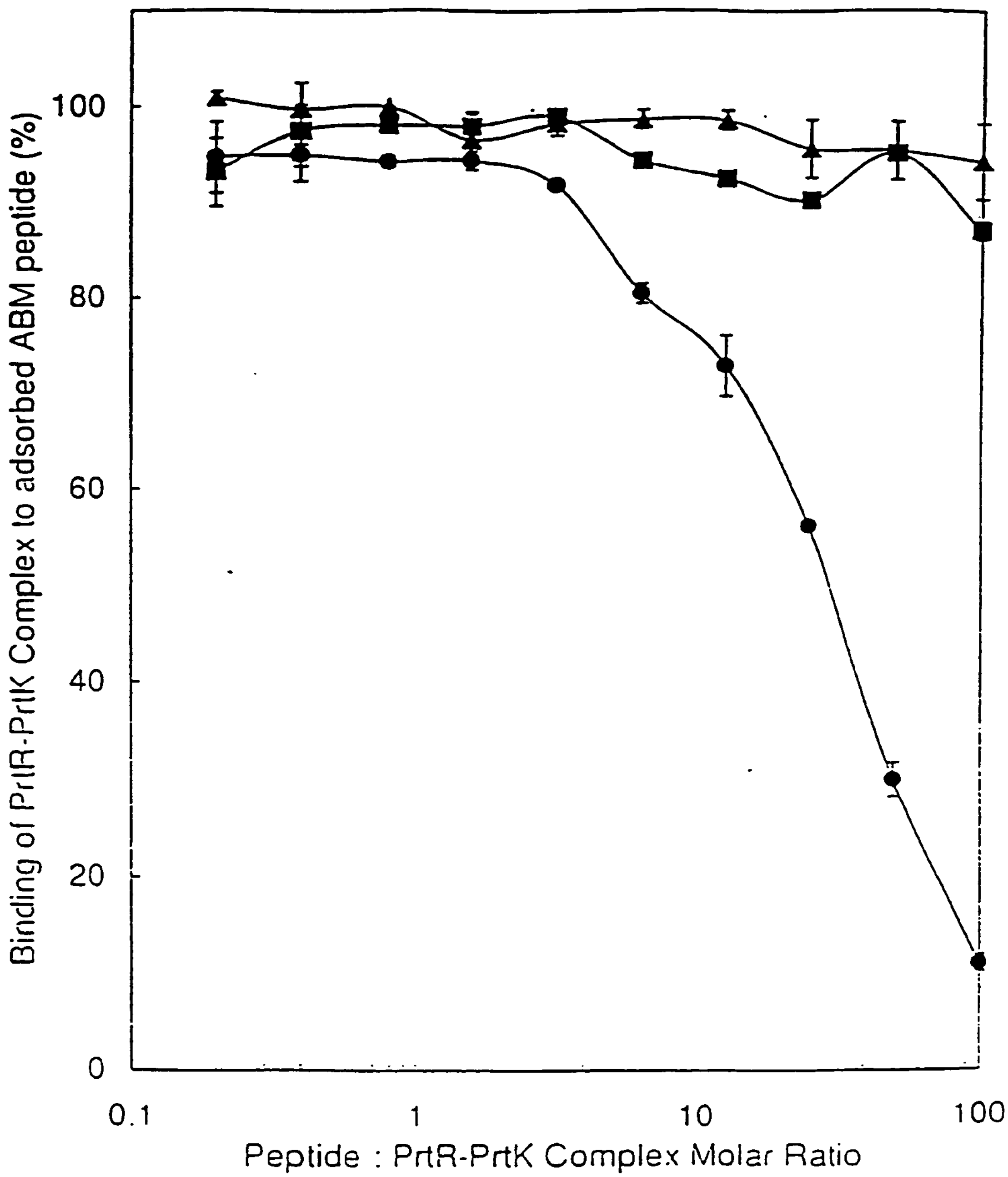

Binding of PrtR-PrtK Complex to adsorbed ABM peptide (%)
100
80
60
40
20
0
0.1
1
10
100
Peptide : PrtR-PrtK Complex Molar Ratio

Figure 10

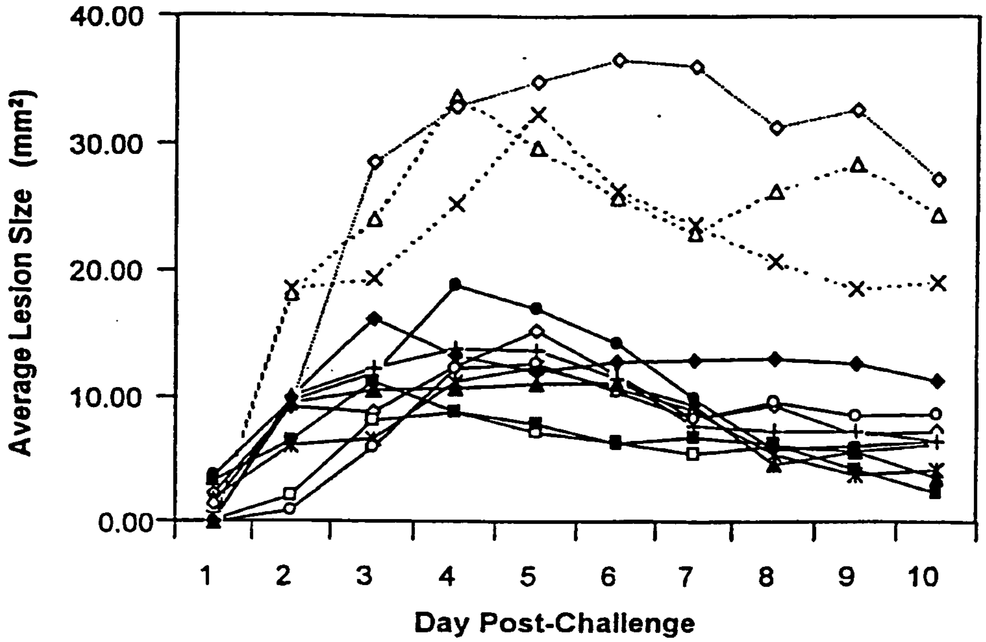

40.00
30.00
20.00
10.00
0.00
Average Lesion Size (mm²)
1
2
3
4
5
6
7
8
9
10
Day Post-Challenge

Figure 11

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00311

A.	CLASSIFICATION OF SUBJECT MATTER
Int Cl[6]:	C07K 7/08, 14/195, 16/12; A61K 39/02, 39/40; G01N 33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC as above

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN: CAS ONLINE: SEQUENCE SEARCH
STN: CAS ONLINE: KEYWORD SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 536 497 (EVANS, R. T. et al.) 16 July 1996 See whole document, especially column 3 line 35 to column 4 line 16, Columns 5-6 and Example III	1-14
P, Y	WO 97/16542 (REYNOLDS, EC et al.) 9 May 1997 See whole document	1-14
Y	Clinical and Experimental Immunology, vol. 110(2) 1997, pages 285-291, Kelly CG et al, "The relationship between colonization and haemagglutination inhibiting and B cell epitopes of Porphyromonis gingivalis" See whole document	1-14

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 June 1998	17 JUN 1998

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	O. L. CHAI Telephone No.: (02) 6283 2482

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00311

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Chemical Abstract, No. 123:112732 Ogawa Tanohiko, "Peptide containing amino acid sequence of fimbrial protein of Porphyromonis gingivalis and use thereof" & WO 95/09181	
P, X	WO 97/34629 (POTEMPA, J et al) 25 September 1997 See whole document	1-14
P, A	WO 97/36923 (REYNOLDS, EC et al.) 9 October 1997 See whole document	1-14
P, X	Microbiology, 1997, vol. 143, pages 2485-2495, Bhogal P.S. et al., "A cell-associated protein complex of Porphyromonis gingivalis W50 composed of Arg-and Lys-specific cysteine proteinases and adhesins" See whole document	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 98/00311

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	9716542	AU	72673/96		
WO	9736923	AU	21452/97		
WO	9734629	AU	24221/97		
WO	9509181	EP	726276	JP	7097395

END OF ANNEX

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00311

Information on patent family members

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WO	9716542	AU	72673/96		
WO	9736923	AU	21452/97		
WO	9734629	AU	24221/97		
WO	9509181	EP	726276	JP	7097395

END OF ANNEX